50th Anniversary of the discovery of Burkitt Lymphoma

February 25-27, 2008 Kampala, UGANDA

International Conference on Burkitt Lymphoma and Related Lymphoproliferative Disorders

Abstracts

Organized in association with the
Makerere University (Kampala) and the
European Association of Hematopathology

Journal of Hematopathology
Volume 1 (2008)
DOI 10.1007/s12308-008-0001-0

Chairmen of the Organizing Committee

Prof. Lorenzo Leoncini,
Department of Pathology and Human Oncology
University of Siena
Siena-Italy
leoncinil@unisi.it

Prof. Harald Stein,
Institute of Pathology
Campus Benjamin Franklin
Charité University Medicine
Berlin-Germany
harald.stein@charite.de

International Scientific Committee

Othieno Abinya, Nairobi, Kenya
James O. Armitage, Omaha, USA
Gabriel Bimenya, Kampala, Uganda
Franco Cavalli, Bellinzona, Switzerland
Volker Diehl, Cologne, Germany
Sandra J. Horning, Stanford, USA
Elaine S. Jaffe, Bethesda, USA
Lorenzo Leoncini, Siena, Italy
Ian Magrath, Brussels, Belgium
Walter Mwanda, Nairobi, Kenya
Stefano Pileri, Bologna, Italy
Martine Raphael, Paris, France
Alan Rickinson, Birmingham, UK
Nelson Sewankambo, Kampala, Uganda
Harald Stein, Berlin, Germany
Lorenz Trümper, Göttingen, Germany

Local Scientific Committee

Henry Wabinga, Kampala, Uganda
Robert Lukande, Kampala, Uganda
Bessie Byakika, Nairobi, Kenya
Jackson Orem, Kampala, Uganda
Charles R. Majinge, Mwanza, Tanzania
James Tumwine, Kampala, Uganda
Birabwa Male, Kampala, Uganda
Lynette Tumwine, Kampala, Uganda
Joshua Nyagol, Nairobi, Kenya
Eoku Yuventine, Kampala, Uganda

ABSTRACTS

BURKITT LYMPHOMA: THE EARLY YEARS.

Dennis Wright, Emeritus Professor of Pathology

University of Southampton, UK

The first clinical records of Burkitt lymphoma (BL) are found in the Mengo Hospital case-notes of Sir Albert Cook at the beginning of the 20th century. Sporadic reports of tumours resembling BL, but often reported as atypical neuroblastomas, appeared in publications from Africa in the first half of the century. Denis Burkitt's seminal publication in 1957 not only described the clinical features of this tumour but showed that the jaw tumours and the visceral tumours were part of the same disease entity. Discussions with George Oettle of the South African Institute for Medical Research, in which it became apparent that the disease had not been recognised in South Africa, stimulated Denis to investigate its distribution in Africa. Using leaflets illustrating the distinctive clinical features of the tumour he contacted medical centres across Africa. The results of this survey, together with the findings of his famous safari through East and Central Africa showed that the tumour was restricted to the "wet topics". This gave rise to the hypothesis that the tumour might be caused by a mosquito borne virus. The observation of space time clustering and epidemic drift in the West Nile District appeared to lend further support to this hypothesis. Following an intensive search for this virus Tony Epstein and his colleagues later discovered the Epstein Barr virus in biopsy samples of BL sent from Uganda. Since this virus has a worldwide distribution it is now thought that the "wet tropics" distribution of BL is related to holoendemic malaria acting as a co-factor in the aetiology of the tumour.

In the 1950's and early 1960's there was no effective treatment for BL in Uganda. Surgery was sometimes attempted but with such a rapidly growing, widely disseminated disease such treatment was futile and radiotherapy was not available locally. Burkitt, with no previous experience of chemotherapy, developed a simple single drug regime using cyclophosphamide. Responses to this therapy were usually dramatic and in some cases led to long term remission.

The term Burkitt tumour's (subsequently changed to Burkitt's lymphoma) was adopted at an international meeting on African Lymphoma in recognition of Burkitt's pioneering contributions to the clinical studies, epidemiology and treatment of this tumour. At a later international meeting held in Washington the majority of delegates agreed that BL should be defined by its morphology (cytology and histology). Two members of the group, however, were of the opinion that clinico-anatomical features were an essential part of the definition. Time has shown that both groups were correct insofar as BL can be defined on morphology but clinico-anatomical features are required to differentiate between endemic, sporadic and AIDS-related BL.

HOW DENIS DISCOVERED THE LYMPHOMA

David Allbrook, Emeritus Professor

University of Western Australia

I was Senior Lecturer in Anatomy, 1952-58 at Makerere College when Denis Burkitt used to bring interesting patients for discussion to the weekly Mulago Hospital clinical meetings. At these Mulago and Makerere medical staff discussed Burkitt's clinical demonstrations of these children he brought in from his surgical safaris. But it was Denis who perceived a pattern of disease and realised that these various tumours might be a single clinical entity. I remember one evening in 1958, just before we were leaving for the USA, he asked me to the Burkitt house on Mulago hill to show me a series of homunculi drawings he had made of the various tumour sites in these children. They were lying on the floor. He asked my opinion „as a medical scientist, because I am only a surgeon"!! (I can't remember my reply- though I probably agreed with him..) In 1961 I returned to Makerere from Washington University, St Louis, Missouri (I had been learning electronmicroscopy to set up a unit at Makerere.) I met a very excited Denis in Mulago Hospital car park,"We have a cure and it's in a little wayside flower- the Madagascan periwinkle., thank God!" I recall the loaded car as he and colleagues left our Makerere home for his amazing pan-African safari,which was another of his amazing field researches in geographic medicine. I think it was funded by a small MRC grant. Then I vividly remember being at a seminal meeting in the bone store of the Anatomy Department at Makerere when Denis demonstrated his geographic findings to Sir Harold Himsworth (UK's MRC Secretary), Alex Haddow (Virus Research Institute, Entebbe) and several others on a huge map of Africa spread on a lab bench. I remember the intense discussion and that Himsworth backed the concept of an insect born vector initiating a virus born cancer. For the historical record and your interest, my talk describes these and other memories of my friend Denis Burkitt and about the important steps he contributed to our present understanding of lymphoproliferative disorders diseases. As they are from my memory (which may be at fault in its detailed time sequences after 50 years,) I must disclaim absolute accuracy of the record.

THE WHO CLASSIFICATION OF BURKITT LYMPHOMA

Elaine S. Jaffe, M.D.

Hematopathology Section, Laboratory of Pathology,
National Cancer Institute, USA

The definition of Burkitt lymphoma (BL) in the WHO classification defines a homogeneous morphological and molecular entity, although there is some variation in the clinical presentation and epidemiological features among the clinical variants of BL. Three clinical variants are recognized: Endemic BL, Sporadic BL, and immunodeficiency-associated BL, mainly associated with HIV-infection. The association with EBV varies among the variants, being highest in endemic BL, and lowest in sporadic BL. Additionally, the proportion of EBV-positive cases corresponds to other epidemiological factors, including the average age of seroconversion in the population and socio-economic status in the population.

The histological variants of BL include: classical BL, atypical BL, and BL with plasmacytoid differentiation. The latter category is closely linked to EBV-positivity and is often HIV-associated. Atypical BL and classical BL have not been distinguishable using gene expression profiling studies, and the presence of slightly atypical cytology does not appear to have any biological or clinical impact. The current WHO classification of BL has a homogeneous immunophenotype, with expression of CD20, CD10, BCL-6, absence of Bcl-2, and a proliferation rate of close to 100% of the neoplastic cells. Using gene expression profiling cases carrying both BCL2/JH and C-MYC translocations have profiled as BL, but clinically double-hit cases have a very different prognosis, and should be distinguished in clinical trials from classical BL.

EPIDEMIOLOGY OF BURKITT LYMPHOMA

EPIDEMIOLOGY OF ENDEMIC BURKITT'S LYMPHOMA IN KENYA

R. Rochford[1], O. Sumba[2], E. Piriou[1], A. Asito[2], J. Rainey[3], P. Rosenbaum1, R. Ploutz-Snyder1

[1]SUNY Upstate Medical University, Syracuse NY, USA; [2]Kenya Medical Research Institute, Kisumu, Kenya; [3]CDC, Atlanta, GA, USA

The question of why Burkitt's lymphoma (BL) is endemic in equatorial Africa has intrigued scientists since the first clinical description of this cancer by Denis Burkitt. Investigation of the geographic restriction demonstrated that BL occurred primarily in regions where there was sustained and intense exposure to holoendemic malaria. We began our studies in Kenya by revisiting the question of whether malaria transmission intensity and BL incident rates were linked. Using a large sample size, stable BL incident rate estimates from a 10-year case study, and more specific malaria endemicity definitions at a district level of analysis, we found that BL rates were 3.5 times greater in regions with chronic and intense malaria transmission intensity than in regions with no or sporadic malaria transmission. To further localize BL cases within Nyanza Province Kenya where the overall risk for BL is high, medical records for all BL cases diagnosed between 1999 and 2004 at Nyanza Provincial Hospital were abstracted to determine the place of residence. Spatial analysis of BL cases within Nyanza Province revealed that BL cases

were not evenly distributed. Interestingly, we found one significant low-risk BL cluster (p = 0.001) and two significant high-risk clusters (p = 0.001). Spatial clustering of BL cases in a high-malaria transmission region suggests that there are additional environmental factors in the etiology of BL. Current studies are examining differences in plasma selenium – an essential micronutrient – between the high and low-BL risk regions in Nyanza Province. Other ongoing studies are examining how infection with Epstein-Barr virus (EBV) early in life alters viral children is being followed from birth through 3 years of age and EBV viral load, antibody levels, B-cell phenotype and EBV-specific T cell responses are being measured. Analysis at 12 months of age revealed that children living in a region with holoendemic malaria were infected with EBV earlier in life, B cell phenotypes were significantly different depending on malaria exposure and EBV specific T cell responses to lytic peptides corresponded with EBV infection. A model for the etiology of BL based on our studies and the studies of others will be presented.

MALARIA-INDUCED DYSREGULATION OF EBV-SPECIFIC T CELL IMMUNITY IN THE ETIOLOGY OF ENDEMIC BURKITT LYMPHOMA

Ann M. Moormann[1], Kiprotich Chelimo[2], Kevin N. Heller[3], Peter Odada Sumba[2], Paula Embury[1], Robert Ploutz-Snyder[4], Juliana Otieno[5], Margaret O'Dour[5], Christian Munz[3] and Rosemary Rochford[4]

[1]Case Western Reserve University, Cleveland, OH, USA; [2]Kenya Medical Research Institute, Kisumu, Kenya; [3]The Rockefeller University, New York NY, USA; [4]SUNY Upstate Medical University, Syracuse, NY, USA; [5]Kenya Ministry of Health, Kisumu, Kenya.

Why children in Equatorial Africa are at high risk for endemic Burkitt lymphoma (BL) has been linked to both early-age Epstein Barr Virus (EBV) infection and holoendemic *Plasmodium falciparum* malaria exposure. The importance of cytotoxic T cells in controlling EBV infections has been well established and it has long been hypothesized that EBV immunity is suppressed as a consequence of repeated malaria infections. However, the means by which malaria orchestrates deficiencies in anti-EBV immunity that result in tumorigenesis have not been so well explained. Our investigations of healthy children with divergent malaria exposure have demonstrated that malaria-induced T cell dysfunction is age-dependent, transient, EBV-specific, and differentially affects EBV-specific T cell memory subsets. The paradox however is that EBV-specific CD8+ IFN-γ responses are primarily targeted to EBV latent antigens other than Epstein-Barr nuclear antigen 1 (EBNA1) while BL tumors expresses only EBNA1. Our investigations of children diagnosed with BL at Nyanza Provincial General Hospital in Kenya show that there is a select loss of the EBNA1-specific CD4+ IFN-γ response in BL patients. EBNA1-specific CD4+ T cells suppress tumor growth in a BL mouse model suggesting that the absence of IFN-γ response to EBNA1 in BL patients allows the emergence of the malignant clone. Furthermore, children with BL maintain IgG1 dominated EBNA1-specific antibody responses similar to healthy children suggesting a loss of IFN-γ secreting EBNA1-specific T cells as opposed to a defect in priming or Th1 polarization. CD8-mediated T cell deficiencies were not observed in BL children for other EBV lytic and latent antigens or for a recombinant blood stage malaria protein, merozoite surface protein 1 (MSP1) which is primarily mediated by CD4+ T cells. The causal mechanisms by which malaria could alter the maintenance of immunity to EBV have led us to postulate a more comprehensive model for eBL pathogenesis.

A ROLE FOR ARBOVIRUSES IN ENDEMIC BURKITT'S LYMPHOMA.

C. A. van den Bosch, MB, BS.,MSc., MD, DCH

Institution: Health Protection Agency, UK

Recent advances in virology, parasitology and immunology have helped to elucidate the important contributions of the Epstein-Barr virus and heavy malarial infection to lymphomagenesis in African endemic Burkitt's lymphoma. However, these infections cannot explain the shifting foci and space-time case clusters of the lymphoma that have been recorded.
Theoretical considerations and epidemiological data raise the possibility that these phenomena may be associated with arboviral epidemics and that they can better explain certain aspects of the epidemiology of the tumour than can the Epstein-Barr virus and malaria. Data from Malawi also suggest that infection with one or more arboviruses is implicated in the crucial later stages of lymphomagenesis.
An increased incidence, and space-time case clusters of endemic Burkitt's lymphoma were observed during the course of an epidemic of the arbovirus, Chikungunya Fever, in Malawi. Cases of Burkitt's lymphoma showed evidence of recent infection with Chikungunya virus and other arboviruses at time of first admission. Lymphoma cases were significantly more likely to be seropositive for Chikungunya viral antibodies than controls and many Burkitt's Lymphoma cases seroconverted for antibodies to this virus during their first admission. These observations support an association between arboviral infection and the late stages of development of endemic Burkitt' s lymphoma. Plant extracts with tumour-promoting factors could also participate in lymphomagenesis.

ANTIBODIES AGAINST MALARIA AND EPSTEIN BARR VIRUS IN CHILDHOOD BURKITT LYMPHOMA: CASE-CONTROL STUDIES IN UGANDA AND MALAWI.

Lucy M Carpenter[1], Robert Newton2, Delphine Casabonne[3], Nora Mutalima[1], Elizabeth Molyneux[4], John Ziegler[5], Sam Mbulaiteye[5], Edward Mbidde[5], Henry Wabinga[5], Harold Jaffe[1], Valerie Beral[3]

[1]Department of Public Health, University of Oxford, UK; [2]Epidemiology and Genetics Unit, Department of Health Sciences, University of York, UK; [3]Cancer Epidemiology Unit, University of Oxford, UK; [4]Department of Paediatrics, College of Medcine, Blantyr, Malawi; [5]Uganda Cancer Institute and Makerere University Medical School, Kampala, Uganda.

Burkitt lymphoma, a childhood tumour common in parts of sub-Saharan Africa has been associated directly with Epstein Barr Virus (EBV), based on studies involving individual children, and indirectly with malaria, based on patterns of population prevalence. We have examined antibodies to both EBV and malaria in individual children diagnosed with this cancer in Uganda and Malawi. In both studies, we performed case-control analyses of HIV-seronegative children (≤ 15 years) admitted to hospital. Cases were diagnosed with Burkitt lymphoma and controls with non-malignant conditions or non-lymphatic cancers. Interviews were conducted and serological samples collected which, when possible, were tested for both EBV and malaria. Adjusted odds ratios (OR) for Burkitt lymphoma were estimated using unconditional logistic regression adjusting for sex, age, residential district, household income and tribe. In the Uganda study, mean age of cases was 7 years and 61% were male. Compared to controls, cases were more likely to be reported having received more frequent treatment for malaria in the past year (OR=2.0; P=0.001) and less likely to be living in a home where insecticides were used (OR=0.2; P<0.0001). Odds of Burkitt lymphoma in children increased with increasing antibody levels against EBV (P<0.0001) and malaria (P=0.05). Findings were similar for children residing in districts close to the capital city and in remote areas. Compared with children with low antibody levels against both EBV and malaria, children with raised levels against both were five times more likely to have Burkitt lymphoma (OR=5.0; P=0.003). Results obtained from the study conducted in Malawi are similar. Our findings suggest that EBV and malaria may act synergistically in the pathogenesis of childhood Burkitt lymphoma. Malaria prevention measures have the potential to also prevent this childhood cancer.

EFFECTS OF DIVERGENT P.FALCIPARUM MALARIA TRANSMISSION DYNAMICS ON B CELL HOMEOSTASIS IN CHILDREN FROM WESTERN KENYA.

A.Amolo[1,2], E.Piriou[3], P.0. Sumba[1], Z.W, Ng ang a[2], A.M.Moormann[4], and R.Rochford[3]

[1]Kenyan Medical Research Institute, Center for Global Health Research, Kisumu, Kenya; [2]Jomo Kenyatta University of Agriculture and Technology; [3]Department of Microbiology and Immunology, SUNY Upstate Medical University, Syracuse, NY; [4]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, USA

Chronic or acute P.falciparum malaria has been associated with perturbation of peripheral B cell subsets, B cell activation, and an increased risk for endemic Burkitt lymphoma. It has been postulated that these aberrancies reflect chronic antigenic stimulation within the context of chronic P.falciparum infection. To test this hypothesis we measured the expression of differentiation markers on peripheral blood B cells from one year old children from two geographically proximate districts; Kisumu district which is a malaria endemic area and Nandi that experiences unstable malaria transmission. Children from Kisumu district showed statistically significant increased B cell frequencies (mean, 21%) compared to those from Nandi district (mean,

16%). Children from Kisumu and Nandi districts had higher frequencies of nad've B cells (CD19+IgD+, mean, 82 versus 81% respectively) B cells and low frequencies of memory B cells (CD19+CD27+, mean of 6% versus 6% respectively). However, the frequencies of IgD+ CD27+memory were significantly increased in children from Nandi district (mean, 6%) compared to Kisumu district (mean, 4%).
Of note, the population of immature transitional B cells (CD19+IgD+CD10+) was significantly increased in children from Kisumu district compared to Nandi district (mean, 31.9 % versus mean 25.8%, respectively). The prevalence of immature transitional B cells in peripheral circulation raises the possibility that B cell dysfunction due to P.falciparum infection may either result in elevation of a population of B cell susceptible to chromosomal translocation or Epstein-Barr virus infection, which may explain the emergence of B cell malignancies like endemic Burkitt s lymphoma in endemic malaria settings.

SELENIUM LEVELS AND RISK FOR ENDEMIC BURKITT'S LYMPHOMA IN WESTERN KENYA.

Peter Odada Sumba[1], Ephantus Kabiru[2], Michael Opiyo[1], Chelimo Kiprotich[1], Alloys S.S. Orago[2], Paula Rosenbaum[3], Ann M.Moormann[4], Rosemary Rochford[3]

[1]Kenya Medical Research Institute, Kisumu, Kenya; [2]Kenyatta University, Nairobi, Kenya; [3]SUNY Upstate Medical School, Syracuse, New York, USA; [4]Case Western Reserve University, Cleveland, Ohio, USA.

Endemic Burkitt's lymphoma (eBL) is the most prevalent pediatric cancer in Equatorial Africa. The etiology of this lymphoma is multi-factorial involving early-age infection with Epstein Barr virus (EBV) and frequent exposure to *Plasmodium falciparum* malaria. Since these co-infections are common in African children living in malaria endemic areas, other eBL risk factors are being investigated. One potential co-factor could be selenium deficiency. Selenium is an essential micronutrient and is an integral component of the antioxidant enzyme glutathione peroxidase (GPx). Many populations worldwide exhibit selenium deficiencies but little is known about selenium deficiency within African populations. Moreover, there is emerging data to suggest the selenium deficiency potentiates viral infections. We hypothesized that deficiencies in selenium could contribute to increased risk for eBL by decreasing host ability to control EBV infection. To test this hypothesis, a cross sectional survey was conducted in children living in a region of Western Kenya that have higher than expected and in a region where lower than expected incident rate of eBL (Rainey et al., 2007, Int J.Cancer, 120:121). Blood was collected from 145 children ages 1 to 11 years, to determine selenium levels using Plasma GPx enzyme immunoassay for selenium GPX. We found that children living in a low-risk area for BL had a mean selenium level of 3.74 µg/dl (n=78) while children living in a high-risk area for BL had a mean selenium level of 2.44 µg/dl (n=67). These values were compared using a t-test for equality of means and the differences were highly significant ($p<0.0001$). Our data demonstrates that there is evidence that children living in a high risk region for eBL have significantly less selenium GPx than children living a low-risk region for eBL but more studies are needed to determine what impact selenium deficiency has on immune function.

COMMON SINGLE NUCLEOTIDE POLYMORPHISMS IN TOLL LIKE RECEPTOR.

Mulama D[1,2], Ptaschinski C[3], Greene J[4], Chelimo K[2], Sumba PO[2], Miller K[4], Ng ang a Z[1], Vulule J[2], Rochford R[3] and Moormann AM[4]

[1]Jomo Kenyatta University of Agriculture and Technology, Kenya; [2]Kenya Medical Research Institute, Kisumu, Kenya; [3]Sunny Upstate Medical University, Syracuse, NY, USA; [4]Case Western Reserve University, Cleveland, OH, USA

Endemic Burkitt s lymphoma (eBL) is the most prevalent paediatric cancer in equatorial Africa. Although an epidemiological link exists between eBL, early childhood Epstein-Barr virus (EBV) infection and holoendemic *Plasmodium falciparum* malaria transmission, the mechanisms by which these pathogens interact to increase the risk of eBL remain largely unknown. Malaria-induced immunosuppression of EBV-specific T cell immunosurveillance has been suggested as a mechanism that increases a child s risk of developing eBL.
A novel line of investigation into eBL etiology lies in understanding innate immune responses to these co-infections and their influence on the development of adaptive immunity. Recent studies implicate single nucleotide polymorphisms (SNPs) in toll-like receptors (TLR) 4 and 9 in susceptibility to severe clinical malaria. However the role of innate immunity in eBL pathogenesis has yet to be determined. Kenyan children (n=264) from regions with divergent malaria endemicity and children diagnosed with eBL (n=204) were screened for the frequencies of TLR9 SNPs (T/C-1486, T/C-1174, G/A1174 and G/A2848) determined by multiplex ligation detection reaction; and TLR4 SNP (Asp299Gly) determined by real timequantitative PCR (RTQ-PCR). EBV viral load, measured by RTQ-PCR amplification of DNA extracted from whole blood, was also compared in these three groups of children stratified by TLR polymorphism. There was no significant association between TLR9 or TLR4 SNP allele frequency and eBL. However, a borderline association of EBV viral load with the TLR9 T/C-1486 SNP was observed at a significance level of 0.08. This suggests that innate immunity may play a role in determining viral set points and therefore may belong in the mechanistic pathway leading to eBL. Future studies are warranted to investigate the functional role of TLR in controlling persistent viral infections and if TLR ligands contributed by malaria co-infections modulate antiviral immunity.

COMPARISON OF EBV STRAINS IN PERIPHERAL BLOOD AND SALIVA OF MOTHER-CHILD PAIRS IN A BURKITT'S LYMPHOMA ENDEMIC REGION OF WESTERN KENYA.

Namuyenga E.T[1], Piriou E[2],Odada P.K[2], Moorman A[3], Ofulla A[1],Vulule J[2], Rochford R[4]

[1]Maseno University, Maseno, Kenya, [2]Kenya Medical Research Institute, Kisumu Kenya, [3]Case Western Reserve University, Cleveland OH USA, [4]SUNY Upstate Medical University, Syracuse New York USA,

There are 2 strains of EBV, EBV Type 1 and Type 2 that exhibit both phenotypic and genotypic differences. Type 1 is the dominant strain in US and Europe while a relatively equal distribution of Type 1 and 2 has been reported in a select number of African countries. There is little information on the prevalence of these two strains in children living in a region at risk for endemic Burkitt s lymphoma. The goal of this study was to determine the prevalence of EBV Type 1 and Type 2 in children and their mothers. Since EBV can be detected in both saliva and blood, we also wanted to compare the differences in virus type between these compartments. In a pilot study, nineteen mother/child pairs were recruited from a region in Western Kenya. For 12 groups, samples were collected from more than one child in the family. PCR primers were used to amplify a region of the EBNA3C gene that can distinguish between the two strains based on the size of the PCR product. DNA was extracted from peripheral blood and saliva and PCR amplification was done on all samples. EBV type 1 and 2 are identified based on length differences within the EBNA3C gene. EBV DNA was detected in the saliva of 46.7% of the mothers and 57.1% of the children. In the blood, EBV DNA was detected in 63.2% of the mothers and 57.9% of children. When we examined the type of EBV strain in saliva in the children, we found in 40.7% were Type 1, 11.1% were Type 2 and 7.4% were Type 1 and 2. In contrast, we observed more mixed infections in the blood where 20.6% were Type 1, 38.2% Type 1 and 2, and in 41.2% no EBV was detected. Comparison of the type of EBV found in mothers and children showed only 37.5% match in strain in saliva but 83.3% match in the EBV strain detected in the blood. In summary, we observed a high percentage of children co-infected with both EBV Type 1 and 2 and the numbers found were much higher than reported for healthy adults in the US. In addition, the information on strain prevalence and transmission patterns will help us better understand the epidemiology of EBV and the potential role of EBV strains in the etiology of eBL.

THE BURKITT TUMOR PROJECT IN ACCRA, GHANA

Robert J. Biggar, MD, Francis Nkrumah and Janet Neequaye U.

State Serum Institute, Copenhagen, Denmark; Ghana Medical School, Accra, Ghana .

In 1969, the US National Cancer Institute established a parallel Burkitt Tumor Project (BTP) at Korle Bu Hospital, the University of Ghana's teaching hospital in Accra, Ghana. Its initial mission was to confirm elsewhere what was being reported in Uganda. However, it soon became critical to Burkitt lymphoma (BL) studies as Uganda became increasingly affected by political turmoil. Active NCI participation lasted until 1979, and local leadership was soon overtaken by the emerging AIDS epidemic. Coverage included mainly the south half of Ghana, from which about 30-40 patients per year were referred for a total evaluation of more than 1,000 patients.

During its years, the Ghana Burkitt Tumor Project published 35 papers on BL. Important observations contrasting with Uganda were the increasing proportion of abdominal rather than facial BL, failure to see a clear-cut relationship to malaria, an HLA Dr7 association, and lack of geographic or familial clustering. A major research focus to understand the natural history of EBV, accepted as being causally important in BL. These studies first established the late infancy/first year) EBV infection pattern in Africa and a lack of clinical symptoms or haematological changes associated with early childhood infection. The observations, now text book standards, are basic to our knowledge of EBV infection. There was no relationship between EBV and malaria antibody titers, a still controversial issue.

MALARIA, HIV AND MALIGNANT LYMPHOMAS IN EAST AND CENTRAL AFRICA AND IN GERMANY.

Schmauz R

Institut für Pathologie, Papenburg and Universität zu Lübeck, Germany

Burkitt's lymphoma (BL), in the up-dated Kiel classification, is a malignant lymphoma (ML) of high-grade malignancy, and associated with both falciparum malaria (FMA) and HIV. A synopsis of own collaborative studies is given assessing such relationships in the remainder of ML. Four different areas are compared, namely two in Uganda during the years 1966-1973 with marked and little FMA. The third area was Rwanda 1979-1987 during the rise of the AIDS-epidemic and without FMA, and the fourth Oberbergisches Land near Cologne during 1991-1993 without FMA and with AIDS in negligible frequency. The cases came from the Kampala Cancer Registry, and the Pathology Departments in Butare and in Gummersbach. Histological review was according to the up-dated Kiel classification and always with assistance of a senior hematopathologist. Proportional frequencies of BL were 52, 28, 9 and 1%, respectively, and for all types of high-grade malignant lymphomas 86, 62, 53 and 40%. For aggressive Hodgkin's lymphomas the values were 75, 60, 64 and 38%, and for high-grade Non-Burkitt, Non-Hodgkin's lymphomas (NBNHL) 70, 44, 45 and 30%. Conclusions: In areas endemic for FMA aggressive/high-grade lymphoid malignancies other than BL are also relatively more common although the difference is not as marked as in BL, and in AIDS-afflicted Rwanda a mild increase of these lymphomas appears possible. Among single types of NBNHL immunoblastic and immunocytic lymphomas were seen most frequently in Rwanda. By contrast, in the other three areas centroblastic and centroblastic/-cytic types were leading. – Other yet unexplained findings of interest were the low relative frequencies of T-cell lymphomas observed in Africa in comparison to Germany with values of 3, 3, 5 and 20%, and the absence of cases of immunocytoma in the very large material from Uganda. – Efforts are under way to include for comparison recent data from an area where both FMA and AIDS are prevalent.

DEVELOPMENT OF EBV-SPECIFIC IMMUNITY DURING THE FIRST YEAR OF LIFE IN CHILDREN WITH DIVERGENT EXPOSURE TO PLASMODIUM FALCIPARUM.

Erwan Piriou[1,2], Amolo Asito[1], Michael Opiyo[1], Chelimo Kiprotich[1], Peter Odada Sumba[1], Ann Moormann[3], Nancy Fiore[2], Robert Ploutz-Snyder[2], Rosemary Rochford[2]

[1]Kenya Medical Research Institute, Kisumu, Kenya; [2]SUNY Upstate Medical University, Syracuse NY, USA; [3]Case Western Reserve University, Cleveland OH, USA

It is hypothesized that P. falciparum induced activation of EBV-infected B cells and suppression of antigen-specific immune responses play an essential role in the pathogenesis of endemic Burkitt's lymphoma. To determine how the development of EBV-specific immunity is affected in children repeatedly infected with P. falciparum, 2 groups of children are being followed longitudinally from birth through 3 years of age. The first group is from an area where P. falciparum is holoendemic (Kisumu District, Kenya), whereas the second group is from an area with sporadic P. falciparum transmission (Nandi District, Kenya). EBV DNA load in whole blood, as well as EBV (VCA, EBNA1, EAd, Zta) and P falciparum (AMA1, MSP1p19, CSP1, LSA1)-specific antibody levels, were determined at monthly intervals during the first year. EBV-specific T cells were enumerated by IFNγ ELISPOT at 12 months of age. By 12 months of age, 33.8% of children from Kisumu and 20.4% of children from district were infected with EBV as determined by detection of EBV DNA. The mean age of infection was 9.5 months versus 10 months in Kisumu vs. Nandi, respectively. Correspondingly, at 12 months of age, 34% and 21% of children had T-cell responses to a pool of lytic EBV epitopes in Kisumu and Nandi, respectively ($p<0.05$). In contrast, the frequency of T cell responses to a pool of latent epitopes was not significantly different between the Kisumu (30%) and Nandi groups (25%). EBV- and P falciparum –specific antibodies are currently being measured using a multiplexed suspension bead array. These first results suggest that more children living in a region with holoendemic malaria are infected with EBV earlier in life and that EBV specific T cell responses to lytic peptides correspond with EBV viral load. Analysis of later time points will help us better understand how the degree of exposure to P. falciparum affects the development of EBV-specific immunity and the ability to control EBV-infected cells.

SINGLE NUCLEOTIDE POLYMORPHISMS IN TOLL LIKE RECEPTOR 4 & 9 ARE NOT ASSOCIATED WITH ENDEMIC BURKITT LYMPHOMA LYMPHOMAGENESIS.

Mulama D[1,2], Ptaschinski C[4], Greene J[3], Chelimo K[2], Amolo A[2], P. O. Sumba[2], Ng ang a Z[1], Vulule J[2], Rochford R[4] and Moormann AM[3]

[1]Kenyatta University, Kenya; [2]Kenya Medical Research Institute, Kisumu, Kenya; [3]Case Western Reserve University, Cleveland OH, USA, [4]SUNY Upstate Medical University, Syracuse NY, USA

Endemic Burkitt s lymphoma (eBL) is the most prevalent pediatric tumor in equatorial Africa where malaria is holoendemic. Although strong epidemiological link exists between early childhood EBV and chronic P. falciparum infection with eBL, the mechanisms by which these ubiquitous microbes interact to increase the risk of tumorigenesis are unknown. Plasmodium falciparum stimulates host immune cells via toll-like receptors especially TLR4 and 9. Polymorphisms in these receptors have been associated with increased susceptibility to human diseases due to altered innate immune responses. In order to determine if these polymorphisms influence eBL risk, the frequencies of four common TLR9 SNPs (T/C-1486, T/C-1174, G/A1174 and G/A2848) and the one TLR4 SNPs (Asp299Gly) were examined in 494 children residing in two regions of western Kenya differing in malaria transmission dynamics and an eBL population. TLR4 SNP was determined by R-T PCR while TLR9 genotypes were determined by multiplex LDR. Results demonstrated that there was no association between TLR9 or TLR4 SNP genotype or allele frequency and history of malaria exposure or risk of eBL ($p> 0.05$). What was however interesting in these populations was the high percentages of heterozygous individuals for any genotype for all of the SNPs. This study highlights the fact that apart from malaria-induced immunosuppression of T cell immunity to EBV there may
be a role for the innate immune response in the etiology of eBL. Future studies are warranted to investigate how malaria infections influence innate immune responses to EBV as a putative mechanism in eBL lymphomagenesis.

IMPACT OF EBV ON BURKITT LYMPHOMA

EPSTEIN-BARR VIRUS IN BURKITT'S LYMPHOMA: HISTORICAL ASPECTS AND INTERACTION OF PERSISTING EBV DNA WITH ENDOGENOUS HUMAN RETROVIRUSES/ HERV

Harald zur Hausen

Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany.

Fifty years ago, in 1958 and subsequently in 1962, Dennis Burkitt described an African lymphoma as a malignancy frequently affecting primarily the jaw region of children aged 5-12 years. The occurrence of this tumor within specific climatic zones characterized by holoendemic transmission of Plasmodium falciparum-caused malaria resulted in his speculation that it may have an infectious origin, possibly transmitted by an arthropod vector. The subsequent development of suspension tissue cultures of cells derived from this lymphoma, independently by Puvertaft and Epstein, permitted a more careful characterization of the tumor tissue. Epstein and his co-workers described in 1964 the presence of herpes virus-like particles in occasional tissue culture cells derived from this tumor. An immunofluorescent test developed by Werner and Gertrude Henle in Philadelphia permitted a further characterization of these particles, now labelled Epstein-Barr virus (EBV), and some of their biological properties. They showed that EBV was unrelated to other human pathogenic herpesviruses, that Burkitt's lymphoma patients revealed elevated antibody levels against this virus, that EBV is able to immortalize normal human lymphocytes and that it represents the causative agent of infectious mononucleosis.

In subsequent studies, Old and co-workers revealed highly elevated antibody titers to EBV also in nasopharyngeal carcinoma (NPC) patients. My own group demonstrated between 1970 and 1973 the presence of EBV DNA in non-virus producing Burkitt's lymphoma cells, as well as in primary biopsies of African Burkitt's lymphoma and nasopharyngeal cancer biopsies. In addition, we showed that the viral DNA persisted in NPC carcinoma cells and not in the commonly present lymphatic infiltrates. The subsequent demonstration of a lymphoma-like disease after inoculation of EBV into squirrel or owl monkeys underlined the role of EBV as a tumor virus, in fact the first human virus consistently found in specific human malignancies.

Within the following years the molecular structure of the EBV genome was unravelled, as well as varying expression patterns of the viral genome in different forms of genome latency. Viral DNA was also demonstrated in human immunoblastomas, arising under conditions of prolonged immunosuppression, in a significant percentage of Hodgkin's lymphomas, in a subset of T-cell lymphomas, and in approximately 10% gastric cancers. This will be briefly summarized. In spite of a detailed knowledge of viral genome structure and viral gene expression patterns, up to today a number of questions still remain open. They concern in part the preferential geographic distribution of the endemic form of Burkitt's lymphoma in equatorial Africa and of NPC in South East Asia, but also the requirement of EBV genome persistence in maintaining the malignant phenotype of the tumor cells. Indeed, evidence is accumulating that EBV may not be required for continuous growth of the respective tumor cells under cell culture conditions or after xenografting NPC cells into nude mice. In addition, outside the endemic regions a high percentage of histologically identical tumors is devoid of detectable EBV DNA, although EBV-positive and –negative Burkitt's lymphomas share similar chromosomal translocations.

Obviously, there exists a need to explain the different patterns of EBV persistence and to identify common mechanisms in the positive and negative tumors driving the growth pattern into a histological more or less identical direction. Recent reports on the induction of a human endogenous retrovirus (HERV) HERV-K18 supernantigen (*Sutkowski et al.*, Immunity 15: 579, 2001, *Hsiao et al.*, J. Immunol. 177: 2056, 2006) may point to an interesting interaction between EBV infection and HERV sequences that comprise approximately 8% of the human genome.

For these reasons we initiated studies to analyze HERV expression and the expression of HERV-related microRNAs in EBV-infected lymphoblasts and latently EBV-carrying Burkitt's lymphoma cells, as well as in EBV-negative Burkitt's lymphoma cells and in cells of additional leukemic and lymphoma cell lines. In preliminary data we could identify the specific expression of a microRNA in all EBV-infected cells tested thus far. Interestingly, the same microRNA was also highly expressed in EBV-negative Burkitt's lymphoma cells, but not in leukemic and other lymphoma cell lines. The significance of these observations is presently being studied.

THE EVOLVING AND INTERTWINING STORY OF PERSISTENT INFECTION WITH EBV AND THE ORIGINS OF BURKITT'S LYMPHOMA

David A. Thorley-Lawson

Tufts University School of Medicine, Jaharis Building, 150 Harrison Ave. Boston MA, USA

Epstein-Barr virus was first discovered in the tumor cells of endemic Burkitt's lymphoma. The subsequent discovery that EBV encodes several oncogenes, was able to transform human B lymphocytes in culture and was found in several other tumors lent support to the idea that EBV was the first human oncogenic virus to be discovered. However it was subsequently found that EBV expressed different patterns of latent proteins in different tumor types and most surprisingly that none of EBVs oncogenic proteins were expressed in BL. The role of EBV as an oncogenic virus became even more mystifying when it was discovered that:

1. There was an EBV negative form of BL and that the unifying defect in BL was a translocated, deregulated c-myc oncogene not the presence of EBV.
2. >95% of the human population harbor EBV for life without pathogenic consequences.
3. The virus persists in resting memory B cells which, as far as we can tell, are completely normal. They are not transformed and the virus is essentially transcriptionally quiescent for latent proteins – a state reminiscent of BL.

This raised the previously unlikely possibility that EBV played no role in BL pathogenesis and was just along for the ride in a memory B cell that happened to become a tumor. Given that 98% of endemic BL are EBV positive this coincidence is hard to explain but how to resolve these contradictions? Answers are now beginning to emerge through the description of a detailed model of EBV persistence that explains the origins of BL and other EBV associated tumors. In this talk I will briefly review the history of EBV as an oncogneic virus in the context of BL, summarize the model of how EBV normally establishes and maintains persistent infection in memory B cells and finally how a molecular understanding of the mechanism of this persistence may explain the role of EBV in BL.

IMMUNE T CELL CONTROL OF EPSTEIN-BARR VIRUS INFECTION AND T CELL TARGETING OF VIRUS-ASSOCIATED TUMOURS

Alan Rickinson

Cancer Research Institute for Cancer Studies University of Birmingham, UK

Epstein-Barr virus (EBV) replicates in permissive cells within the oropharynx and colonises the B cell system through a latent, growth-transforming infection. Primary infection elicits strong CD8+ T cell responses to lytic and latent cycle antigens that then persist for life in the immunocompetent host. Both sets of antigens show marked hierarchies of immunodominance, with focusing of responses against epitopes drawn mainly from the immediate early and some early antigens of lytic cycle and against the EBNA3 nuclear antigens of latent cycle. Though less abundant, EBV-induced CD4+ T cell responses are also becoming well characterised. Interestingly, these show different immunodominance hierarchies, with late lytic cycle antigens and the latent cycle EBNA1 antigen often eliciting the strongest reactivities.

With this a background, it is important to know (i) whether EBV-associated tumours arise in the presence or absence of virus-specific immunity, (ii) what are the most effective responses to harness against the tumour (ii) how best to boost such responses and direct them to the tumour site? The example of Burkitt Lymphoma (BL) is particularly interesting because the tumour cells show restricted viral antigen expression (usually EBNA1 only) and have a defect in MHC class I processing that prevents CD8+ T cell recognition. However BL cells do retain MHC class II processing function and a capacity to present some endogenously expressed antigens to CD4+ T cells. Two immunotherapeutic approaches are being explored in this context. One seeks to target BL using CD4+ T cells specific for EBNA1-derived epitopes. A second exploits a novel population of cytotoxic CD4+ T cells that recognise autologous EBV-transformed B cells but are not EBV-specific. These effectors appear to be directed against cellular target antigens that are up-regulated in EBV-infected (but not mitogen-activated) B cells and are also expressed in BL cell lines of endemic and sporadic origin irrespective of EBV status.

THREE RESTRICTED FORMS OF EPSTEIN-BARR LATENCY COUNTERACTING APOPTOSIS IN C-MYC EXPRESSING BURKITT LYMPHOMA CELLS

Gemma Kelly, Julianna Stylianou, Andrew Bell, Wenbin Wei, Martin Rowe and Alan Rickinson.

CRUK Institute for Cancer Studies, The University of Birmingham, UK

Epstein-Barr virus (EBV) is aetiologically linked with Burkitt Lymphoma (BL) but its contribution to lymphomagenesis, versus that of the chromosomal translocation activating c-myc expression, remains unclear. This is in part because the full virus growth transforming programme that is expressed when EBV infects normal resting B cells, is not expressed in BL. Instead EBV in BL normally exhibits a restricted Latency I form of infection characterised by expression of only one latent antigen EBNA1 from the BamHI Q promoter. Here we describe an endemic BL, Awia, which
uniquely is heterogeneous at the single cell level for EBV gene expression. Analysis of single cell clones of Awia-BL revealed cells displaying three forms of restricted EBV latency: (i) classical Latency I, (ii) Wp-restricted latency expressing EBNAs 1, 3A, 3B, 3C and -LP, and (iii) a novel EBNA2+/LMP1 latency in which all EBNAs including EBNA2 are expressed without the latent membrane proteins LMPs 1 and 2. Comparison with rare EBV-negative clones from the same tumour showed that each form of infection provides the c-myc-expressing BL cells with a specific degree of protection from apoptosis. Microarray analysis was carried out on the isogenic Awia-BL clones to determine the influence of EBV gene expression on cellular transcription. Interestingly it was found that expression of the pro-apoptotic Bcl2 family protein BIM, which has previously been implicated in c-myc induced

apoptosis, was down regulated in the apoptosis resistant BL cells. Our work suggests that EBV acts as an anti-apoptotic rather than a growth-promoting agent in BL by down regulating expression of the cellular BIM protein. In addition microarray analysis on these isogenic Awia-BL clones showed that the EBNA profile influences the differentiation status of the BL cell on the germinal centre to plasmacytoid differentiation pathway. These findings may reflect viral functions that are important for BL pathogenesis and for EBV persistence.

EBV GENOME LOSS FROM ENDEMIC BURKITT LYMPHOMA AND ITS EFFECTS ON CELL PHENOTYPE.

A. Boyce, R. Feederle, H.J. Delecluse, A. Bell, A. Rickinson, G. Kelly.

Cancer Research Institute for cancer studies., University of Birmingham, UK

EBV's growth transforming function appears central to the pathogenesis of post-transplant lymphoproliferative disease. However the virus contribution to Burkitt Lymphoma (BL) remains uncertain since usually only one viral protein (the virus genome maintenance protein EBNA1) is expressed in BL cells. Studies on Akata-BL, an unusual sporadic BL line which spontaneously generates EBV-loss clones in late passage, first suggested EBV s role in BL was anti-apoptotic. To test the generality of this finding, we single cell cloned 11 early passage endemic BL cell lines and obtained EBV genome-negative clones from four lines, two in very early passage (Mutu-BL and Awia-BL), two at passage 40 (Eli-BL and Kem-BL). The propensity for genome loss did not correlate with steady state levels of EBNA1 but was associated with a more unequal segregation of the multiple EBV episomes at mitosis, as visualised by fluorescence in-situ hybridization (FISH). We infer that the inability to rescue EBV-negative clones from other lines reflects the rarity of complete genome loss among their daughter cells rather than any inherent non-viability of such EBV-loss cells. On all four of the above BL backgrounds (as in Akata-BL), EBV-loss clones were consistently more sensitive to a range of apoptosis inducers including anti-IgM cross linking, ionomycin and the neurotransmitter fluoxetine. This association between EBV status and a BL cell s sensitivity to apoptosis could not be explained by any observed difference in expression of the Bcl-2 family member proteins. By microarray analysis, while EBV-positive and EBV-loss clones show very similar cell transcription profiles, some genes are differentially expressed; their relevance to the above differences is being investigated.

EBNA1-SPECIFIC IFN-GAMMA PRODUCING T CELL MEMORY SUBSETS DIFFER WITH AGE.

Kiprotich Chelimo[1], Paula Embury[2], Peter O. Sumba[1], Christian Münz[3], Rosemary Rochford[4] and
Ann M. Moormann[2]

[1]Kenya Medical Research Institute, Kisumu, Kenya; [2]Case Western Reserve University, Cleveland, Ohio, USA; [3]Sunny Upstate Medical University, Syracuse, NY, USA; [4]The Rockefeller University, New York, NY, USA.

The importance of cytotoxic T cells in controlling EBV infections has been established however further investigation into the phenotype of EBV-specific T cell may shed light into the etiology of endemic Burkitt lymphoma (eBL). IFN-gamma; mediates antiviral immunity though the source of this effector function may differ between children and adults, and in those exposed to malaria. T cells can be categorized into subsets based on surface marker expression and ability to secret cytokines in response to specific antigens. The development of immunity to EBV in children co-infected with Plasmodium falciparum malaria may therefore result in altered memory T cell differentiation. We focused our initial investigations on IFN-gamma; T cell responses to EBNA1, the sole viral antigen expressed in eBL tumors. Peripheral blood mononuclear cells were isolated from Kenyan children and adults from a malaria holoendemic area. IFN-gamma; responses were measured by ELISPOT and immunophenotyped by intracellular staining and flow cytometry. As expected, the frequency of IFN-gamma; responses to EBNA1 increased with age coincident with overall T cell maturation. The majority of EBNA1-specific IFN-gamma; T cell responses was generated by CD4+ and CD8+ effector memory T cells (TEM: CD62L-/CD45RA-) however there was a slight but noticeable trend for CD4+ TEM to increase with age while CD8+ TEM decreased. When examining IFN-gamma; T cell responses from RA re-expressing effector memory T cells (TEMRA: CD62L-/CD45RA+) the inverse was observed. EBNA1-specific IFN-gamma; responses from CD4+ TEMRA decreased with age while CD8+ TEMRA increased. These results reveal an age-associated difference in EBNA1-specific T cell memory subsets producing IFN-gamma. Since EBNA1 is processed differently for presentation to CD4+ versus CD8+ T cells these findings suggest a mechanism by which malaria could interfere with the development of immunity to EBV and thereby predispose a child to eBL.

RAF2 BY EPSTEIN BARR VIRUS LATENT MEMBRANE PROTEIN

Ilaria Guasparri, Ethel Cesarman

Weill Cornell Medical College, USA.

A mechanism used by Epstein-Barr virus (EBV) for in vitro transformation of B cells into lymphoblastoid cell lines (LCLs) is activation of the nuclear factor kappa B (NF-954;B)pathway, which is largely mediated by the EBV latent membrane protein 1 (LMP1). LMP1 is co-expressed with a few other EBV latent proteins, including LMP2A, in some AIDS-related diffuse large B cell lymphomas, post-transplantation Lymphoproliferative disorders and Hodgkin's lymphomas. Since inhibition of NF-954;B leads to apoptosis of EBV-infected lymphoblastoid and lymphoma cell lines, we sought to determine whether LMP1 alone, or in combination with other viral proteins, is responsible for initiating NF-954;B activation in these cells, thereby playing a role in cell survival. Using RNA interference, we found that suppression of either LMP1 or LMP2A results in inhibition of basal NF-κ;B and induction of apoptosis in LCL and lymphoma cell lines. Simultaneous elimination of both LMP1 and LMP2A showed no significant additive or synergistic effect. Studies to elucidate the mechanistic basis for this observation revealed that suppression of LMP2A results in decreased transcription of TRAF2, which in turn is essential for LMP1-mediated activation of NF-954;B in LCL and EBV-infected lymphoma cell lines, while other TRAFs are not. Our data contrasts with previous studies showing that transfected LMP1 can signal in the absence of LMP2A or TRAF2, and demonstrate the requirement for both LMP2A and TRAF2 in naturally infected lymphoma cells and LCLs. These results also support LMP1, LMP2A and TRAF2 as potential therapeutic targets in a subset of EBV-associated lymphoid malignancies.

DNA METHYLATION PATTERN OF THE LMP1 GENE IN THE EPSTEIN-BARR VIRUS (EBV) AND ITS ASSOCIATION WITH LYMPHOPROLIFERATIVE DISORDERS.

Filipe Jacinto, Sónia Guedes-Melo, Augustin Fernandez-Fernandez, Pilar López, Irene Pino, Cecília Rosales, Biola Javierre, Miguel Angel Pires and Manel Esteller

Institution: Cancer Epigenetics Laboratory, Molecular Pathology Programme, Spanish National Cancer Centre (CNIO), Melchor Fernandez Almagro 3, 28029 Madrid, Spain

It has been estimated that 15% of cancer cases are etiologically linked to viral infection; accounting for nearly 1.5 million new cases annually worldwide. One of the viruses most related with carcinogenesis is Epstein-Barr virus (EBV) which is a ubiquitous herpes virus associated with a variety of lymphoid and epithelial tumours, as the nasopharyngeal carcinoma or even gastric cancer (Young, 2004). 90% of the world s adult population is infected by the virus, and it persists in the vast majority of individuals as a lifelong, asymptomatic infection of the B-lymphocyte cells (Gatza, 2005).
Previous studies have shown that EBV expression can be modulated by promoter methylation. Since most parasitic elements that are methylated in the Human Genome never reactivate again, EBV has evolved using CpG promoter hypermethylation to maximize persistence in host cells silencing gene expression. This epigenetic alteration of the viral genome appears to play a crucial role in the regulation of viral gene expression in normal and neoplastic tissue, in the escape of infected cells from immune surveillance, and in the resistance of infected tumour cells to antiviral drugs. Further studies are needed to better understand the biology of this tightly regulated process of EBV gene expression and thus to maximize the therapeutic strategy for the treatment of EBV-associated tumours.
The main aim of this study was to characterize the DNA methylation pattern of the EBV gene LMP1 to better understand the role played by DNA methylation in the biology of the EBV infection in patients with lymphoproliferative disorders associated with the virus and how this epigenetic signature can be used for future therapeutic treatments.

CD34+ CORD BLOOD CELL-TRANSPLANTED MICE AS A MODEL FOR EPSTEIN-BARR VIRUS INFECTION OF THE HUMAN IMMUNE SYSTEM. A MORPHOLOGICAL, IMMUNOPHENOTYPICAL AND MOLECULAR STUDY.

Mario Cocco[1], Cristiana Bellan[1], Roxane Tussiwand[2], Davide Corti[2], Elisabetta Traggiai[2], Stefano Lazzi[1], Susanna Mannucci[1], Lucio Bronz[3], Nazzareno Palummo[1], Piero Tosi[1], Antonio Lanzavecchia[2], Markus G. Manz[2], Lorenzo Leoncini[1]

[1]Department of Human Pathology and Oncology, Division of Pathological Anatomy, University of Siena, Siena, Tuscany, 53100, Italy. [2]Institute for Research in Biomedicine, Bellinzona, CH-6500, Switzerland. [3]Ospedale San Giovanni, Bellinzona, CH-6500, Switzerland.

Recent studies suggest that Epstein-Barr virus (EBV) infects naïve B cells, driving them to differentiate into resting memory B cells via the germinal center reaction. This has been inferred from parallels with the biology of normal B cells but has never been proved experimentally. Rag2-/-γc-/- mice, transplanted as newborns with human CD34+ cord-bood cells, were recently shown to develop human B, T, and dendritic cells, constituting lymphoid organs in situ. Here we used this model to better define the strategy of EBV infection of human B cells in vivo and to compare this with different conditions of EBV infection in humans. Our results support the model of EBV persistence in vivo in cases characterized by follicular hyperplasia and a relatively normal CD4+ and CD8+ T cell distribution. Intriguingly, in cases characterized by nodular and diffuse proliferation with a preponderance of CD8+ T cells, similar to infectious mononucleosis, EBV still infects naïve B cells, but also induces clonal expansion and somatic ongoing mutations, without germinal center reactions. Our results reveal different strategies of EBV infection in B cells, possibly resulting from variations in the host immune response.

EBV ONCOPROTEINS EBNA3A AND EBNA3C FUNCTIONALLY INTERACT TO REPRESS EXPRESSION OF THE TUMOUR-SUPPRESSOR BIM: CLUES TO THE PATHOGENESIS OF BURKITT'S LYMPHOMA (BL)

Emma Anderton, Jade Yee, Kostas Paschos, Paul Smith, Tim Crook, Rob White and Martin J Allday

Department of Virology, Imperial College London, Faculty of Medicine, Norfolk Place, London W2 1PG, UK.

Bim is a pro-apoptotic member of the Bcl-2-family of proteins. The level of Bim is a critical regulator of lymphocyte survival and reduced expression is a major contributor to lymphoproliferative disease in both>mice and humans; Bim is a haploinsufficient tumour-suppressor in B cells. Moreover because it is a specific target in Myc-induced apoptosis Bim is uniquely important in the pathogenesis of BL, since here Myc is always deregulated by gene translocation. EBV induces the continuous proliferation of primary B cells as lymphoblastoid cell lines (LCLs) and if EBV-negative BL-derived cells are infected with EBV, latency-associated viral factors confer resistance to various inducers of apoptosis and dramatically reduce Bim expression. Nuclear proteins EBNA3A and EBNA3C are essential to establish LCLs and they are involved in the resistance of BL cells to cytotoxic treatments. We therefore created using an EBV-BAC system recombinant EBVs from which each (or all) of the EBNA3 genes has been independently deleted and revertant viruses in which the genes have been re-introduced into the viral genome. Infection of EBV-negative BL cells with this panel of EBVs and challenge with various cytotoxic agents revealed that EBNA3A and EBNA3C cooperate as the main determinants of drug-resistance and the down-regulation of Bim transcription. There was no evidence of altered Bim RNA or protein stability and relief of EBV-mediated repression by histone deacetylase (HDAC) inhibitors suggests that epigenetic modifications to chromatin are involved. By epigenetically suppressing the expression of Bim, through the combined action of EBNA3A and EBNA3C, EBV significantly increases the likelihood of B-lymphomagenesis in general and endemic (e)BL in particular. These results may partly explain the selection pressure giving rise to a subset of eBL that retain expression of the EBNA3 proteins and why we find that Myc, p53 and 14ARF can remain non-mutated in these tumours.

IMMUNE ESCAPE IN EBV-POSITIVE BURKITT´S LYMPHOMA

Judith Reschke[1], Martin Schlee[1], Sebastian Grömminger[1],Gerhard Laux[1], Hanna Scheller[1], Madhusudhanan Sukumar[2], Andrea Wilke[2], Armin Gerbitz[2,3], and Georg W. Bornkamm[1]

[1]Institute of Clinical Molecular Biology and Tumor Genetics, GSF-Research Center for Environment and Health, Marchioninistr. 25, D81377 Munich, [2]Med. Klinik III, LMU University Hospital and GSF-Clinical Cooperation Group, D3177 Munich, Germany, [3]present address: Med. Klinik III, Charité Berlin, Benjamin Franklin Campus, D12200 Berlin, Germany

Burkitt´s lymphoma is not recognized by cytotoxic T cells specific for EBV antigens. We have shown in a conditional in vitro system mimicking essential steps of the pathogenesis of BL that c-MYC contributes actively to immune escape by negatively regulating the NF-κB and the IFN response. Studying the IFN response in BL cell lines in more detail, we have found that EBV-positive, but not EBV-negative BL cells are resistant to IFNγ, yet they are susceptible to IFNα/β. In EBV-loss variants of BL cells the IFNγ response is not restored ruling out a viral gene product as mediator of IFNγ resistance. Genetic defects in the IFNγ pathway could also be excluded by demonstrating that group III BL cells (as well as EBV-immortalized cells) are invariably sensitive to IFNγ, whereas group I cells are resistant. These data point to an epigenetic mechanism leading to silencing of the IFNγ signalling pathway in EBV-positive BL group I cells. No difference was observed in the expression of the IFNγ receptor by FACS and quantitative RT-PCR analysis. A significant difference was, however, found when JAK2 expression was studied. EBV-negative BL cells consistently expressed JAK2, albeit at low level, whereas the majority of IFNγ resistant EBV-positive group I BL lines lacked JAK2 expression virtually completely.Reintroduction of JAK2 restored IFNγ responsiveness at least partially. Our data suggest that immune escape in BL cells to viral antigens is mediated by two mechanisms: the immunesuppressive action of c-MYC and the unresponsiveness of BL cells to IFNγ. We propose a model in which cross priming of phagocytosed BL cells may stimulate IFNγ production by activated T cells. IFNγ secreted by activated T cells may thus overcome the immune suppressive action of c-MYC. According to this model IFNγ resistance may be a necessary step in the development of EBV-positive BL in vivo. We finally present a Igλ-myc transgenic mouse model that allows to address this question experimentally.

EPSTEIN-BARR VIRUS AND OTHER MALIGNANCIES: OUR EXPERIENCE

Buda Francesco[1],Argona Pietro[2],Lagostina Fernando[3], Martellotti Miranda

[1]Internal Medicine-Medical Oncology-City of Udine Hospital, Udine,Italy; [2]Clinacal Research Laboratory and Pathology, S,Vito Hospital,Pordenone,It aly; [3]Dpt of Pathology,University of Messina;

In addition to Burkitt's lymphoma ad undifferentiated nasopharyngeal carcinoma the association of the EBV and other malignancies is emerging. We have identified EBV-DNA by Souther blot hybridization analysis in malignant limphoproliferations in nodes obtained from patients with Wiskott-Aldrich syndrome, Cheidiak-Higashi syndrome and in a well differentiated secondary B-cell lymphoma arising in a patient with hairy cell leukaemia. We have determined the clonality of these lymphoproliferations by identifying rearranged heavy chain immunoglobulin sequences. Hybridization with a recombinant DNA probe containing the immunoglobulin heavy chain joining region identified multiple DNA fragments in DNA from Wiskott-Aldrich and Chediak-Higashi syndrome indicating the expansion of several clonal B-cell populations. The secondary malignancy in hairy cell leukemia was monoclonal. These results are similar to the malignant progression in EBV proliferations in organ transplant recipients or severely immunodeficient individuals in wich there is proliferation of several EBV-infected clones and eventual emergence of a monoclonal population. The emergence of EBV-infected lymphoproliferation in Chediak-Higashi or hairy cell leukemia, suggest a role for natural killer cells(NKC)in controlling EBV-transformed lymphocytes.

EPSTEIN BARR VIRUS WITH HUMAN CANCER

Buda Francesco

Internal Medicine-Medical Oncology-City of Udine Hospital, Udine, Italy

The Epstein Barr virus is a ubiquitous human herpes virus and can be associated with silent or symptomatic primary infection such a infectious mononucleosis, a self limiting lymphoproliferative disease characterized by polyclonal proliferation of EBV infected B cells. EBV is associated with malignant lymphoproliferation such as African Burkitt's lymphoma, B-cell lymphomas arising in immune deficient patients including HIV-positive individuals, and various types of peripheral T cell lymphomas in apparently immunocompetent individuals of Asian and Western population. Moreover, EBV is firmly associated with certain epithelial neoplasias such as low differentiated nasopharyngeal carcinoma and undifferentiated carcinoma of the salivary gland in Greebìnladic Eskimos. These tumours are usually of the lymphoepithelioma-type, i.e. the neoplastic cells are associated with a prominent lymphoid stroma. The possible association between EBV and certain other epithelial tumours are presently less clear. Importantly, in the EBV-associated human tumours mentioned, EBV genomes and EBV gene products have been demonstrated by in situ methods (nucleic acid hybridization, immunohistology)to be localized to the tumour cells and are not easily found in adjacent non neoplastic lymphoid or epithelia cells.

INFLUENCE OF HIV ON BURKITT LYMPHOMA

CLINICAL AND PATHOLOGICAL DATA ON HIV-RELATED BURKITT LYMPHOMA.

Martine Raphaël, Caroline Besson, Alex Duval, Chrystèle Bilhou-Nabera, Fanny Ménard, Hélène Antoine-Poirel, Christian Gisselbrecht, Jean Gabarre

Univ Paris-Sud, Inserm U802, AP-HP Hôpital Bicêtre,
Le Kremlin-Bicêtre F94270, France

Three clinical variants of Burkitt lymphoma (BL) are recognised by the WHO classification: endemic BL, sporadic BL and immunodeficiency associated BL. The latter is one of the main histologic subtype of HIV-related lymphoma. There is a significant relationship between the subtype of lymphoma and the HIV status, BL occuring in less severely immunodeficient patients. Most BL patients present with advanced clinical stage. Bulky disease with a high tumour burden, infiltration of the bone marrow but unfrequent peripheral blood involvement, extracerebral nervous system and liver are clinical features, LDH is markedly elevated. At histologic level, classical BL and the plasmacytoid differentiation morphological variant showing abundant basophilic cytoplasm and eccentric nucleus are described. There is significant difference of CD4+ cell count between the two variants: classical BL having a significant higher CD4+ cell count than the plasmacytoid variant. Epstein-Barr virus (EBV) is present in 30% of classic BL and in 50-70% of plasmacytoid differentiation BL. There are no significant differences at genetic level between the two morphological variants of BL involving C-myc oncogene and some additional genetic abnormalities.
Thus, the characteristics of HIV-related BL are the most aggressive clinical features but associated with a better HIV disease status.

HIGH-GRADE LYMPHOMA IN PATIENTS WITH AIDS-RELATED NON-HODGKIN'S LYMPHOMA (AR-NHL) IN EAST AFRICA: OBSERVATIONS FROM A PROOF-OF-CONCEPT PHASE II TRIAL WITH ORAL CHEMOTHERAPY.

[1,4]Remick SC, [2]Orem J, [3]Mwanda W, [2]Katongole-Mbidde E, [4]Meyerson H, and [5]Ayers L

[1]West Virginia University Morgantown, WV, USA; [2]Uganda Cancer Institute, Kampala, Uganda; [3]Kenyatta National Hospital, Nairobi, Kenya; [4]Case Western Reserve University, Cleveland, OH, USA; and [5]The Ohio State University, Columbus, OH, USA.

Introduction: The emergence of Burkitt's-like lymphoma in homosexual men heralded the onset of the AIDS epidemic and the recognition that NHL was seen in increased incidence in HIV-infected patients. At the outset of the epidemic high-grade tumors clearly predominated. As the epidemic evolved intermediate-grade lesions were more commonly encountered. In Africa, detailed histopathological and molecular characterization of NHL has not been reported. A retrospective series of Burkitt's lymphoma in Kenya (Int J Cancer 92:687, 2001) reported an approximate 3-fold increase incidence in adult patients coincident with the AIDS epidemic and median survival of 15 weeks.
Patients and Methods: From among 257 prospectively screened patients with AR-NHL we treated 49 with dose-modified oral chemotherapy. This regimen had a very acceptable 6% mortality rate, 78% objective response rate, and median survival of 12 mos. Pathology was reviewed and tumor phenotype was analyzed using a tissue microarray (TMA) method.
Results: Thirty-two of 49 (65%) patients had pathological material submitted for review. Thirty-one samples were sufficient for histopathology review and 29 samples suitable for attempted TMA phenotyping. Overall histologic review, 29 tumors were classified, as high-grade NHL not otherwise specified including 3 cases that were originally assessed in East Africa as intermediate to high-grade. Another 20 tumors were regarded as intermediate-grade including one low and one low-intermediate grade originally assessed in East Africa. Immunophenotyping delineated 4 B-cell lymphomas, 6 diffuse large-cell lymphomas (DLBCL), 1 anaplastic large cell lymphoma, 1 Hodgkin's disease, 4 lymphoblastic lymphomas including one T-cell derived, 4 Burkitt's lymphoma (3 EBER+), 2 plasmablastic lymphomas (both EBER+) and 7 without sufficient numbers of intact cells (necrosis). C-myc gene rearrangements were confirmed in 2 Burkitt's (2 had no probe signal) and in one DLBCL. Six other samples had normal c-myc arrangements and the remainder had no probe signal. Survival was increased in patients with intermediate vs. high-grade tumors (p=0.013).
Conclusions: High-grade lymphoma appears to predominate in the backdrop of AIDS in East Africa. The diversity of lymphoma in this setting is greater than anticipated suggesting molecular testing for as a worthwhile future capacity building endeavor. NHL phenotyping and molecular profiles of lymphoma encountered in HIV infection in Africa may yield important clues to improved therapy. [Supported in part by NIH grants nos.: CA83528, CA70081, and CA066531.]

EVIDENCE FOR AN ASSOCIATION BETWEEN INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS-1 (HIV) AND BURKITT LYMPHOMA FROM CASE-CONTROL STUDIES IN UGANDA AND MALAWI.

Robert Newton[1], Nora Mutalima[2], Elizabeth Molyneux[3], Delphine Casabonne[4], John Ziegler[5], Sam Mbulaiteye[6], Edward Mbidde[7], Henry Wabinga[8], Harold Jaffe[2], Valerie Beral[4], Lucy M Carpenter[2]

[1]Epidemiology and Genetics Unit, Dept. Health Sciences, University of York, UK; [2]Department of Public Health, University of Oxford, UK; [3]College of Medicine, Blantyre, Malawi; [4]Cancer Epidemiology Unit, University of Oxford, UK; [5]UCSF, California, USA; [6]NCI, USA; [7]UVRI, Uganda; [8]Uganda Cancer Institute & Makerere University Medical School, Kampala, Uganda.

Studies from Europe and the USA indicate a substantial excess risk of the sporadic form of Burkitt lymphoma among HIV infected children, but this has been little studied in Africa, where the classical form of disease, first described by Denis Burkitt, is relatively common. We investigated the impact of infection with HIV on the risk of Burkitt lymphoma among children aged 15 years or less in two case-control studies conducted in Kampala, Uganda and in Blantyre, Malawi. In both studies, cases were children diagnosed with Burkitt lymphoma and controls were children diagnosed with non-malignant conditions or cancers other than those known to be HIV-associated (i.e. excluding Kaposi s sarcoma and other haematological cancers). Interviews were conducted and serological samples collected and, when possible, tested for antibodies against HIV. Odds ratios (OR) for Burkitt lymphoma were estimated using unconditional logistic regression adjusting for sex, age and residential district. In Uganda 10/33 cases were HIV seropositive, compared to 11/190 controls (adjusted OR=7.5, 95% confidence interval (CI) 2.8-20.1). In Malawi, 9/146 cases were HIV seropositive, compared to 2/93 controls (adjusted OR=12.4, 95% CI 1.3-116.2). In conclusion, infection with HIV increases the risk of Burkitt lymphoma among children in Africa, although the magnitude of the excess risk appears less than for the sporadic form of the tumour found among HIV infected children in Europe and the USA.

IMMUNITY IN PERSONS WITH AIDS-RELATED BURKITT LYMPHOMA COMPARED TO OTHER LYMPHOID MALIGNANCIES.

Robert J. Biggar (State Serum Institute, Copenhagen, Denmark);
Anil Chaturvedi, Sam Mbulaiteye, James Goedert and Eric Engels (National Cancer Institute, Bethesda, Maryland USA)

State Serum Institute, DK and NCI, USA

Background. Burkitt lymphoma (BL) incidence in persons with AIDS is declining as therapies improve, although not so much as diffuse large B cell types or Kaposi sarcoma (KS). We examined risk of the AIDS-related cancers and Hodgkin lymphoma by CD4 count. Methods. PWA registered in 12 regional US AIDS registries were linked to local cancer registry data. Among those entering follow-up cancer-free, cancer incidence was determined for 4-27 months from AIDS onset in 1990-1995 and 1996-2002. We used proportional hazards models to assess the relationship between CD4 count at AIDS onset and cancer incidence.
Results. Among 325,516 adult PWA, the incidence of KS and overall NHL was inversely associated with CD4 counts in both time-periods. In 1996-2002, for each 50 CD4 cells/uL decline, the hazard ratio (HR) for KS was 1.40 (1.33-1.50). Among NHL subtypes, the relationships with CD4 counts varied markedly. For BL, the hazard ratio with each 50 CD4 cells/uL decline was 0.93 (0.81-1.06) whereas for central nervous system (CNS) NHL the HR was 1.75 (95% CI: 1.64-1.88), and for non-CNS diffuse large B cell lymphoma, it was 1.12 (1.04 to 1.20). Hodgkin lymphoma risk decreased significantly in severely immunosuppressed patients, and shifted phenotype, with nodular sclerosing types being hardly seen in persons with <50 CD4 cells. In 1990-95, the same relationships were seen with even stronger hazard ratios.
Conclusions. BL was not CD4-sensitive, and Hodgkin lymphoma risk actually decreased with profound immunosuppression, in contrast to patters for other NHLs. These variations indicate a complex pathogenesis underlying each malignancy. They are particular surprising for BL, which has been considered an immunologically sensitive, virally-related tumour. Events increasing BL in persons with HIV/AIDS appear to start before profound immunosuppression occurs and, we speculate, might be related to antigen drive rather than immunodeficiency.

ROLE OF CHROMATIN REMODELLING MEDIATED BY THE HIV-1 TAT PROTEIN IN THE GENESIS OF HIV-ASSOCIATED MALIGNANCIES.

Giulia De Falco, Giovanna Cerino, Eleonora Leucci, Joshua Nyagol, Mario Cocco, Anna Onnis, Piero Tosi and Lorenzo Leoncini

Dept. Human Pathology and Oncology, University of Siena, Italy

The incidence of non-Hodkin's lymphoma (NHL) is greatly increased in HIV-infected individuals. Malignant lymphoma is the second most common neoplasm (after Kaposi's sarcoma) that occurs in association with acquired immunodeficiency syndrome (AIDS). The vast majority of neoplasms are clinically aggressive, monoclonal B-cell neoplasms that exhibit Burkitt's, immunoblastic, or large cell lymphoma histopathology. Although the molecular mechanism underlying HIV-mediated transformation is not clearly understood, the Tat protein of HIV is a likely candidate to contribute to tumour pathogenesis in HIV-infected patients. Tat protein is an early non-structural protein necessary for virus replication, which is secreted by infected cells and taken up by uninfected cells. Extensive evidence indicates that Tat is a cofactor in the development of AIDS-related neoplasms and the protein has also been found to have an oncogenic role in vitro and in vivo. There is experimental evidence to suggest a potential role of Tat-mediated chemotaxis and invasion in the pathogenesis of AIDS-related malignancies. Deregulation of cellular genes and functions by Tat can also cause abnormalities that may contribute to AIDS pathogenesis and to the development of AIDS-associated disorders. The molecular mechanism underlying Tat's pleotropic activity may include the generation of functional heterodimers of Tat with cell cycle proteins. In particular, Tat protein of HIV has also recently been shown to physically interact with the RB2/p130 tumour suppressor gene product and E2F4, resulting in uncontrolled cell proliferation. The interaction of Tat with cell cycle regulatory proteins alone may not be sufficient for neoplastic transformation in vivo and other cofactors may be required.
Another mechanism, through which Tat may influence HIV-mediated transformation, is by hyper-activation of transcription by interacting with chromatin remodelling complexes. Though viral transcription is fully dependent upon host cellular factors and the state of host activation, recent findings indicate a complex interplay between viral proteins and host transcription regulatory machineries, including histone deacetylases (HLACs), histone acetyltransferases (HATs), cyclin-dependent kinases (CDKs), and histone metyltransferases (HMTs). The chromatin structure presents a significant barrier to transcription. These modifications and alterations of chromatin structure increase DNA accessibility to transcription factors and activators, thus promoting transcription initiation and efficient elongation. Many reports in the last several years have linked Tat transactivation to chromatin remodelling in vitro and in vivo.
The aim of our study is to investigate whether Tat-mediated chromatin remodelling may have a role in HIV-associated transformation. The results of our work will be discussed during the meeting.

T-HELPER 1 VERSUS T-HELPER 2 LYMPHOCYTE IMMUNODYSREGULATION IS THE CENTRAL FACTOR IN GENESIS OF BURKITT LYMPHOMA: HYPOTHESIS

Joseph Lubega

University Hospital of North Staffordshire, Stoke-on-Trent, UK

Background. The HIV epidemic has challenged our previous understanding of endemic Burkitt's lymphoma. Despite the strong association of Burkitt's lymphoma and HIV infection in the Developed world, and against previous postulations that the cancer is due to immunosupression among African children, the HIV epidemic in the Malaria belt has not been associated with a corresponding increase in incidence of childhood Burkitt's lymphoma. Even outside the context of HIV infection, there is substantial evidence for a strong but skewed immune response towards a TH2 response in genesis of Burkitt lymphoma.
Presentation of the hypothesis. Rather than a global and/or profound immunosupression, the final common pathway in genesis of Burkitt's lymphoma is the dysregulation of theimmune response towards a TH2 response dominated by B-lymphocytes, and the concomitant suppression of the TH1 cell-mediated immune surveillance, driven by various viral/parasitic/bacterial infections.
Testing the hypothesis. Case control studies comparing TH2 and TH1 immune responses in Burkitt lymphoma of different etiological types (sporadic, HIV-related, endemic and post-transplant) to demonstrate significant dominance of TH2 immune response in presence of poor CMI response as a common factor. Immunological profiling to evaluate differences between immune states that are associated (such as recurrent Malaria infection) and those that are not associated (such as severe protein-energy malnutrition) with Burkitt lymphoma. Prospective cohorts profiling chronology of immunological events leading to Burkitt lymphoma in children with EBV infection.
Implications of the hypothesis. The dysregulation of the immune response may be the missing link in our understanding of Burkitt lymphomagenesis. This will provide possibilities for determination of risk and for control of development of malignancy in individuals/populations exposed to the relevant infections.

HIV-1 TAT INCREASES MICRO VESSELS DENSITY INDEPENDENTLY OF VEGF EXPRESSION IN AIDS-RELATED DIFFUSE LARGE B-CELL AND BURKITT'S LYMPHOMAS.

Nyagol J[1], De Falco G[1], Palummo N[1], Shaheen S[2], Bellan C[1], Lazzi S[1], Spina D[1], Giordano A[1,3], Leoncini L[1]

[1]Department of Human Pathology and Oncology, University of Siena-Italy; [2]Department of Pathology, Aga Khan University Hospital, Nairobi-Kenya; 3Sbarro Institute of Molecular Medicine, Temple University, Philadelphia, USA.

Angiogenic switch marks the beginning of tumour's strategy to acquire an independent blood supply. In some subtypes of non-Hodgkin's lymphomas, higher local vascular endothelial growth factor expression has been correlated with increased micro vessel density. However, this local VEGF expression seems to be higher only in tumours with elevated expression of the receptors of the growth factor, suggesting an autocrine growth-promoting feed back loop. Several studies have indicated that VEGFRs are also targeted by the Tat protein expressed by HIV-1-infected cells. Given the similarity of the basic region of Tat to angiogenic factors (bFGF, VEGF), Tat is thought to mimic these proteins and binds to their receptors. In this study, we sought to evaluate the role of HIV-1 Tat protein in regulating the level of VEGF expression and micro vessel density in AIDS-related diffuse large B-cell and Burkitt's lymphomas. Using luciferase assay, we found out that VEGF promoter was

inactivated in a Burkitt's lymphoma cell line transfected with Tat. Reduced VEGF protein expression in primary HIV-1 positive BL and DLBCL as compared to the negative cases supported the findings of promoter inactivation from the cell lines. Micro vascular density assessed by CD34 expression was, however, higher in HIV-1 positive than in HIV-1 negative tumours. These results suggest that Tat binds to a different site on the VEGFRs, and acts as angiogenic factor independent of VEGF expression. Thus, targeting Tat protein itself and stabilizing transient silencing of VEGF expressions or use of monoclonal antibodies against their receptors in AIDS-associated tumours will open a window for future explorable pathways in management of angiogenic phenotypes in AIDS-associated non-Hodgkin's lymphomas.

PATHOLOGY AND BIOLOGY OF BURKITT LYMPHOMA

MOLECULAR PATHOGENESIS OF BURKITT LYMPHOMA.

Kishor Bhatia

National Cancer Institute, NIH, Bethesda, USA

A sine quo non of Burkitt's lymphoma is the presence of a translocation between the c-myc gene and the IgH gene which is an early and a pivotal event in lymphomagenesis. In addition to this c-myc rearrangement, the features of which vary depending upon geography, sustainance of the tumor phenotype and or progression potentially involves a varaible contribution by EBV as well as accumulation of other genetic events. These lesions include mutations in p53 and c-myc genes as well epigenetic modulation of silencing of other tumor suppressor genes. Additional studies using BL tumor cell lines demonstrate mutiple abberrations in the apoptotic pathway. Some of these very lesions provide tumor specific targets for designing novel therapeutic strategies. The question of the biological and clinical relevance of these molecular lesions has not been fully studied. We hypothesize that variations in the genetic and epigenetic lesions in BL could be responsible for some differences of the clinical features of BL in Africa, including it's heightened chemosensitivity.

C-MYC REGULATION AND THE CONSEQUENCES OF ITS DEREGULATION

Juhong Liu[1], Fedor Kouzine[1], Lawrence Benjamin, Hye-Jung Chung[1], Zuqin Nie[1], Ashutosh Gupta[1,2], and David Levens[1]

1Laboratory of Pathology, Center for Cancer Research, NCI, Bethesda, MD 20892-1500, USA. [2]Department of Physics, University of Maryland College Park, MD

Because *c-myc* translocation with an Ig-locus in is a sine quo non for Burkitt lymphoma, it necessary to understand the function and regulation of MYC in order to explain the pathogenesis and describe the pathology of this disease. MYC is an HLH-bZIP protein that heterodimerizes with MAX and is a master regulator of transcriptional and chromatin activity, regulating the expression of hundreds, if not thousands of targets. While most MYC-targets are up-regulated by the oncogene, some are repressed. Through these targets the influence of MYC is projected physiologically or pathologically upon proliferation, cell growth, apoptosis, differentiation, metabolism, macromolecular synthesis and cell signaling. Thus far, no discrete set of MYC targets have defined a linear pathway for carcinogenesis in Burkitt lymphoma or in other tumors associated with deregulated *c-myc* expression. Whether the pathology associated with MYC results from deregulation of just a few proper targets or from the inappropriate activation of pathologic targets has not been fully resolved. Moreover, what constitutes pathologic *c-myc* activation remains unclear; for example in Burkitt lymphoma MYC levels range from physiological to massively over-expressed.

Recent evidence suggests that physiological *c-myc* expression is held to close tolerances with the potential for untoward consequences from excessive cell-to-cell variation in MYC levels. Many features of the c-myc promoter may be explained (or at least rationalized) as a system to constrain such variation, both at steady state and during induced peaks of c-myc transcription. Because MYC protein and mRNA are normally both sparse and short lived, a hierarchy of systems have evolved to control *c-myc* transcription precisely and accurately in real-time. Though the *c-myc* promoter is robust to inactivation, its physiological operation is sensitive to the conformation and topology of its DNA and to the configuration of its chromatin, rendering the gene susceptible to genetic disturbances.

MYC BREAKPOINTS IN BURKITT LYMPHOMA.

PM Kluin[1], JEJ Guikema[1,2], S Rosati[1], EJ Boerma[1], E Schuuring[1]

[1]Dept. of Pathology and Laboratory Medicine, University Medical Center Groningen, The Netherlands, [2]Department of Molecular Genetics and Microbiology, Program in Immunology and Virology, University of Massachusetts Medical School, Worcester, MA 01655, USA

The t(8;14), or the variants t(8;22) and t(2;8) are hallmark translocations of Burkitt lymphoma (BL). These Ig-MYC breakpoints are found in approximately 75-80%, 10-15% and 5% of all cases and lead to juxtaposition to Ig enhancers and to constitutive over expression of MYC. Somatic mutations and breakpoints within the MYC gene might result into additional deregulation by inactivation of regulatory regions or use of the P3 promoter, and might also interfere with binding of BIM involved in apoptosis. In BL, Ig-MYC breakpoints are primary events; similar translocations but also translocations involving MYC and other partner genes, can occasionally be found in diffuse large B cell lymphoma and during progression of "indolent" lymphomas such as follicular lymphoma. Mapping of MYC breakpoints indicate distinct clustering far 5' or far 3'of MYC in endemic BL and clustering immediately 5'or within MYC in sporadic cases. Recent studies indicate the presence of two smaller breakpoints clusters within MYC itself. Approximately 10% of otherwise characteristic molecular BL, including some endemic BL lack a detectable breakpoint. This might be due to methodological problems or alternative mechanisms leading to MYC over expression. In endemic BL the Ig breakpoint most often involves the VH-JH regions and in sporadic BL mostly the heavy chain switch regions. Both breakpoints are thought to be mediated by aberrant, AID-mediated recombination events in germinal centre B-cells. During normal class switch recombination, DNA double stranded breaks are introduced in Sμ and a downstream switch region, which are subsequently juxtaposed and ligated with deletion of the intervening DNA. In contrast, in almost all sporadic BL switch recombination of the translocated allele affects only one switch region, resulting in a perfect reciprocal translocation. In some cases with a breakpoint downstream of Cμ, IgM is transcribed from this allele and not from the non-translocated IgH allele.

C-MYC NEGATIVE CLASSICAL BURKITT LYMPHOMA: ALTERNATIVE PATHOGENETIC MECHANISMS

Eleonora Leucci[1], Mario Cocco[1], Anna Onnis[1], Patricia van Cleef[2], Cristiana Bellan[1], Anke van Rijk[2], Giulia De Falco[1], Joshua Nyagol[1], Bessie Byakika[3], Stefano Lazz[1], Piero Tosi[1], Johan van Krieken[2], Lorenzo Leoncini[1]

[1]Department of Human Pathology and Oncology, University of Siena, Italy; [2]Department of Pathology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [3]Department of Pathology, The Nairobi Hospital, Nairobi, Kenya

The molecular hallmark of Burkitt lymphoma (BL) is the translocation that place MYC under the control of immunoglobulin gene regulatory elements. However, from the EUROFISH project, aiming at the standardization of FISH procedures in lymphoma diagnosis, emerged that 5 cases out of 35 classic endemic BLs were negative for MYC translocation. Here we investigated the expression of miRNAs directed against c-myc in eBL cases; we found Let-7c to be down-regulated in BLs compared to the reactive lymph node. More interestingly, hsa-mir34b was down-regulated in BL cases negative for MYC translocation. This event might be responsible for c-myc deregulation, since we demonstrated that hsa-mir-34b is able to regulate c-myc. Moreover the finding that c-myc is regulated by this miRNA creates an important regulatory link between p53 and c-myc pathway.

GAIN OF 13q31-q32 IS THE MOST COMMON GENETIC ABERRATION IN PEDIATRIC SPORADIC BURKITT LYMPHOMA.

EG Boerma[1], V Nadarajan[1], JL Robertus[1], P van der Vlies[2], K Kok[2], A Zettl[3], A Rosenwald[3], E Campo[4] , PM Hoogerbrugge[5], JHJM Krieken[6], WA Kamps[7], KH Lam[8], IM Appel[9], SS Dave[10], LM Staudt[10], E Schuuring[1], A van den Berg[1], PM Kluin[1] – *EGB and VN contributed equally

[1]Department of Pathology & [2]Department of Medical Genetics, University Medical Center Groningen, The Netherlands, [3]Institute of Pathology, University of Würzburg, Germany, [4]Department of Pathology and Hematology, Oncology Hospital Clinic, University of Barcelona, Spain, [5]Department of Paediatrics & [6]Department of Pathology, University Medical Center Nijmegen, The Netherlands, [7]Department of Pediatric Oncology, University Medical Center Groningen, [8]Department of Pathology, Erasmus Medical Center, Rotterdam, [9]Dutch Childhood Oncology Group, The Hague, The Netherlands, [10]Metabolism Branch, Division of Cancer Treatment and Diagnosis, NCI, NIH, Bethesda, MD, USA.

Two recent studies show that sporadic Burkitt lymphoma (BL) has a relatively robust gene expression profile. In both studies the profile was mainly based on the analysis of paediatric sporadic BL, which therefore may be considered as a prototype of BL. Without additional array-type gene expression analysis, BL is less easily to diagnose. For example in adult patients BL may be difficult to discern from diffuse large B-cell lymphomas with a similar very high proliferation rate. A better Definition of genetic alterations additional to the hallmark MYC-translocation (with juxtaposition to one of the immunoglobulin loci) might therefore help to improve diagnosis.
Classic and an array based comparative genomic hybridization (CGH) with 6465 BAC/PAC clones were applied on 18 abdominal BL from homogeneously treated paediatric patients. Genomic alterations were compared to gene expression analyses obtained with the Affymetrix U133 plus 2.0 platform.
In 17 out of the 18 BL we found 76 aberrations. Array CGH identified more aberrations than classic CGH. Recurrent segmental gains (n≥3) were observed at 3q29, 7q11, 11q22-q23 and 13q31-q32. Recurrent hemizygous losses (n≥3) involved 1q41, 11q24-qter, 13q21, 13q33-qter, 14q32 and 17p. ACEit analysis pointed out a number of potential target genes including TP53. The most common genetic aberration was gain at 13q31-q32 (n=6) with a minimal common region of 6.9 Mb. The two differentially expressed genes located in this region were c13orf25 and glypican-5
(GPC5). Quantitative RT-PCR of the individual micro-RNAs in the polycistron c13orf25 also showed differential expression levels indicating differences in processing and/or stability of these microRNAs.

BURKITT LYMPHOMA IN UGANDA IN THE THIRD MILLENNIUM: A MORPHOLOGIC AND MOLECULAR APPRAISAL ON TISSUE MICRO-ARRAY.

Cristina Campidell[1] MD[1], Lynnette K. Tumwine MD[2], Simona Righi BS[1], Stefano A. Pileri MD[1]

[1]Chair of Pathology, Unit of Hematopathology, Institute of Hematology and Clinical Oncology L. & A. Serŕgnoli , Bologna University School of Medicine, 40138 Bologna, Italy [2]Department of Pathology, Makerere University Medical School, P.O. Box 7072, Kampala, Uganda

129 routine tissue samples, collected over 10 years at Makerere University, were used for tissue micro-array (TMA) construction. Sections were cut from TMAs and used for Giemsa staining, immunohistochemistry and in situ hybridization. 95 cases were classified as Burkitt s lymphoma. The sites of disease were the abdomen (57%), lymph node (33%), and jaw (10%). Morphologically, BL showed a cohesive and monotonous medium-sized neoplastic infiltrate, with frequent mitotic figures, apoptotic bodies, and starry sky pattern. 43 cases exhibited plasmacytoid differentiation. BL always expressed CD10 and Bcl-6. In all instances but three, Bcl-2 was negative. Proliferation index was close to 100%. Interestingly, CD30 and CD138 were found in 35 and 43 cases, respectively. EBV integration was detected in all instances. As expected, BL was the commonest diagnostic category according to the WHO classification. This result is much higher than what found in a similar study in Kenya but lower than what reported in a case control study in Uganda. This difference might be due to the fact that only a limited number of immunohistochemical markers were used in such studies. BL is a high grade neoplasm that occurs sporadically worldwide, but is endemic in Papua New Guinea and in central Africa. Notably, the phenotypic profile obtained in this study largely confirmed previous reports. A large amount of CD138 and CD30 positive cases was observed. The former finding largely corresponded to the plasmacytoid differentiation observed at microscopic evaluation. On the contrary, the latter was recorded irrespectively of morphology and might reflect the tumorigenesis process. Our findings partly differ from Burkitt's prototypic description and resemble what seen in Western Countries among sero-positive patients. Studies on the HIV status and MYC aberrations are ongoing at present and will be presented at the meeting, along with the possible correlation with the low incidence of jaw involvement.

Id PROTEINS IN BURKITT LYMPHOMA

Annette H. Schmitt-Graeff[1], Michael Müller[2], Hendrik Veelken[3] and Oliver Opitz[2]

Institute of Pathology and comprehensive Cancer Center, University hospital of Freiburg, Germany.

Background: Id proteins (Inhibitor of differentiation/DNA binding) are dominant negative regulators of basic helix-loop-helix transcription factors. Members of the family include Id-1, Id2, Id3, Id4. Their interaction with E-transcription factors blocks E-protein activity and results in a disruption of the differentiation program of various cell types. Id proteins are expressed in normal fetal and adult lymphoid tissues. Until now, the precise function of Id proteins in malignant lymphomas is incompletely assessed.
Methods and results: This prompted us to investigate the expression patterns of Id proteins in lymph node and bone marrow biopsies from 25 patients with sporadic Burkitt lymphoma. We used commercially available Id1, Id2, Id3 and Id4 antibodies and the LSAB detection method. The staining intensity and the immunolocalization (nuclear vs. cytoplasmic) of tumor samples were evaluated and compared to normal lymph nodes and bone marrow specimens. All cases showed a strong predominantly nuclear expression of Id1. Immunolabeling by Id2 and Id2 antibodies revealed both cytoplasmic and nuclear expression patterns. Interestingly, the expression levels were higher in tumor cells disseminated to the bone marrow than in nodal manifestations. Id4 was clearly down-regulated both in nodal disease and in bone marrow trephines.
Conclusions: The high levels of Id1, Id2 and Id2 may be positively associated with the proliferation of Burkitt lymphoma cells and promote the dissemination to extra lymphatic sites, such as the bone marrow. Apparently, Id4 is inactivated in tumour cells. Whether the silencing is due to mutation of the Id4 gene or rather DNA methylation remains to be further investigated. Hypermethylation of the Id4 gene has been reported in EBV transformed B cells. Our data suggest that Id4 may play a role as tumor suppressor gene in Burkitt lymphoma.

FOXP1 IN BURKITT LYMPHOMA PATIENTS CROATIAN EXPIRIENCE.

Petra Kora[1], Ivana Peran[1], Roland A Ventura[2], Slobodanka Ostoji; Koloni[3], Alison H Banham[2], Mara Dominis[1]

[1]Department of Pathology and Cytology, Merkur University Hospital, Zagreb, Croatia, [2]Nuffield Department of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford, UK, [3]Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia

Since 1980, 15 patients with Burkitt or Burkitt-like lymphoma have presented at the University Hospital Merkur in Zagreb, Croatia. Burkitt lymphoma is a very rare tumor in Croatia, not necessarily linked to younger patients. Tumor cells of this aggressive lymphoma are believed to originate from normal germinal centre or post-germinal center B-cells. Recently it has been shown that the FOXP1 transcription factor is an essential transcriptional regulator for early B-cell development. The FOXP1 locus is targeted by recurrent chromosome translocations in both diffuse large B-cell lymphoma and MALT lymphoma, which are thought to de-regulate its expression. FOXP1 also represents a prognostic marker in these malignancies, and in cutaneous B-cell lymphoma, with high-level expression identifying poor prognosis patients. In this study we analyzed 15 Burkitt and Burlitt-like lymphoma patients by immunohistochemistry and detected FOXP1 protein in all cases and in almost all tumor cells. We performed FISH, detecting various FOXP1 gene abnormalities (n=6) and verifying the presence of the IGH/MYC translocation (n=5). The same strong FOXP1 protein expression was observed independently of FOXP1 gene translocation (n=1), additional gene

copy (n=5), or no genetic abnormality, suggesting that these do not influence protein expression levels. Furthermore, we searched for EBV infection (2 cases being positive) and grouped patients according to age, gender or tumor localization and found no correlation with FOXP1 aberrations. In conclusion, we report the first study demonstrating FOXP1 expression in Burkitt lymphoma and suggest that genetic abnormalities do not appear to be the primary mechanism regulating its expression.

BURKITT'S LYMPHOMA: A HISTOPATHOLOGICAL ANALYSIS OF 26 CASES.

Samaila Modupela Omotara A

Ahmadu Bello University Teaching Hospital

Background: Endemic African Burkitt's lymphoma has a predilection for the jaw. However, other sites are increasingly involved in affected children and adolescents.
Aim: To analyze the site distribution of Burkitt's lymphoma.
Materials &Method: A review of all cases of Burkitt's lymphoma diagnosed from tissue specimens seen in Pathology department of Ahmadu Bello University Teaching Hospital, Zaria over a 7year period (2000-2006).
Histology slides stained routinely with Haematoxylin & Eosin were studied. Patients' personal data and clinical information were obtained from records.
Results: Twenty-six cases were analyzed. They accounted for 24.5% of all lymphomas cases and 28.6% of malignant childhood tumours seen within the study period. There were 16 males and 10 females. The age range was 18months to 16years with a mean age of 6.9years for both sexes. Four patients were aged above 11years. Eleven (11) patients presented with jaw masses involving the mandible and maxilla. Of these, five had bilateral jaw masses and four had associated intra-abdominal masses while four patients had associated dental anarchy and proptosis. Eleven (11) of the children presented with abdominal masses while four (4) had orbital swellings and impaired vision. Histologically, all showed diffuse sheets of lymphoblasts having fairly uniform round blue nuclei with scanty cytoplasm interspersed by foamy histiocytes and exhibiting characteristic starry sky appearance. The histology identified specific organs affected in the abdomen as the ovary (5), kidney (2), retroperitoneum (2) and mesentery (2). Cytogenetic analysis to determine if all the cases were endemic was not possible in our resource constraint setting.
Conclusion: Burkitt's lymphoma is still prevalent in our environment and abdominal presentation is common. Adolescents are increasingly affected.

BURKITT LYMPHOMA: ROLE OF pRB2/p130 INACTIVATION DURING LYMPHOMAGENESIS.

Eleonora Leucci1, Giulia De Falco[1,2], Anna Onnis[1], Annalisa Roberti[1,2], Antonella Guzzo[1,2], Lorenzo Leoncini[1] and Antonio Giordano[1,2]

[1]Department of Human Pathology and Oncology, University of Siena, [2]Sbarro Institute for Cancer Research & Molecular Medicine, Biolife Science Building Suite 333, Temple University Philadelphia, PA19122, USA.

Burkitt lymphoma (BL) is a highly aggressive B-cell lymphoma consisting in three variants: endemic, sporadic and immunodeficiency-associated. The molecular hallmark of BL is the over-expression of c-Myc, due to the translocation of this gene in proximity of the immunoglobulin promoter. However, MYC translocations are not specific for BL; in fact, MYC translocations have been reported 5-10% of DLBCL (diffuse large B cell lymphoma) and B lymphoblastic leukaemia/lymphoma. Moreover additional genetic and epigenetic alterations involving particularly p16 and p53 pathways have been described.
We previously demonstrated, that the *RBL2/p130* gene, a member of the retinoblastoma family, is mutated in BL cell lines and primary tumors. As pRb2/p130 controls cell growth, its inactivation may enhance the effect of c-myc over-expression and may contribute to transformation. In this study, we tried to reproduce c-myc over-expression and *RB2/p130* silencing in a human non-transformed lymphoblastoid cell line, to analyze the contribution of these two genes in malignant transformation.
We found that pRb2/p130 silencing cooperates with c-myc over-expression decreasing the percentage of apoptotic cells and speeding up proliferation. Moreover, we demonstrated that pRb2/p130 mutation is not essential for malignant transformation, but is crucial for tumor progression, by conferring to the cells the ability to cross the extra cellular matrix.

CYTOGENETIC ANALYSIS IN DIFFERENTIAL DIAGNOSTICS OF BURKITT LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA.

Obukhova TN, Baryakh EA, Zvonkov EE, Magomedova AU, Kaplanskaya IB, Kremenetskaya AM, Kravchenko SK, Domracheva EV, Vorobjev AI

National Research Center for Hematology, Moscow, Russia

The distinction between Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) is difficult in a substantial number of cases. Nevertheless this distinction is of great importance because these two lymphomas require different treatment strategies. We analyzed 119 patients with aggressive predominantly extranodal highly proliferative mature B-cell lymphomas using morphological, immunohistochemical and cytogenetic assay to establish differential diagnostic criteria. Conventional cytogenetic assay (CCA) was performed in 26 pts, standard FISH in 21 pts, in 72 cases only paraffin-embedded tissue was available for FISH-detection of c-myc rearrangements (c-mycR). By CCA t(8;14)(q24;q32) was revealed in 9 cases and variant t(8;22)(q24;q11) was found in 2 cases.
In 15 pts complex karyotypes were found without c-mycR. By FISH t(8;14) was detected in 43 pts, c-mycR - in 2 cases and in 48 cases c-mycR was not revealed. Group with c-mycR consists of 56 pts: 44 males, 12 females, mean age 22 years. 83% has generalized disease stages, bone marrow Involvement was in 30 %, neuroleukemia in 24%. Extranodal sites of involvement were: ovarium 64%, intestine 43%, liver 35%, kidneys 24%, stomach 21%, facial bones in 2 cases. Atypical morphology was observed in 26% of pts; atypical immunophenotype in 21% ; in 5 cases Ki-67 was < 80%. Group without c-mycR consists of 63 pts: 27 males, 36 females, mean age 47 years. In 89% of cases extranodal tumour localization was observed, however, specific for BL organs involvement was rare: intestine 15%, kidneys 5%, ovarium 0%. We did not revealed pts with bone marrow involvement or neuroleukemia. Typical for BL morphology was found in 10% of pts; 29% of cases were CD10+, Bcl6+, Bcl2-; in 74% Ki-67 was ~100%. We conclude: Typical for BL clinical, morphological and immunohistochemical pattern can be observed in DLBCL. Differential diagnostics between BL and DLBCL is possible only on the basis of cytogenetic analysis.

HSA-MIR-127 IS IMPLICATED IN B CELL DIFFERENTIATION AND ITS EXPRESSION IS ALTERED IN BURKITT'S LYMPHOMA.

E Leucci[1], A Onnis[1], M Cocco[1], V Costanzo[1], G De Falco[1,2], C Bellan[1], L Leoncini[1]

[1]Department of Human Pathology and Oncology, University of Siena, Italy. [2]Sbarro Institute for Cancer Research and Molecular Medicine Center of Biotechnology, Biolife Science Building Suite 333, Temple University Philadelphia, PA 19122, USA.

Burkitt lymphoma (BL) is a highly aggressive B-cell lymphoma that occurs in three clinical variants: endemic, sporadic and immunodeficiency-associated-BL.
Recent data suggests that EBV-positive and EBV-negative BLs have different cells of origin. In particular, immunoglobulin gene mutation analysis, suggests an early centroblast origin for EBV-negative BLs, on the other hand EBV-positive BLs appears to originate from post germinal center cells, in spite of their germinal center phenotype. The appearance of a germinal center phenotype in EBV-positive cells, might result from the activity of deregulated c-Myc, and thus not reflecting the cell of origin of BL, or might derives from a block in B cell terminal differentiation. MiRNAs are a class of small RNAs that regulate gene expression post-transcriptionally. We recently reported a down regulation of a c-Myc-regulating miRNA in Burkitt lymphoma cases lacking MYC translocation. Here we showed that hsa-mir-127 is strongly up regulated only in EBV-positive BL samples. Moreover we showed that it is implicated in physiological B cell differentiation both *in vivo* and *in vitro*. According with the down-regulation of this miRNA during plasma cell differentiation, we also demonstrated that it is able to inhibit in a dose dependent manner BLIMP-1 and XBP-1, resulting in up regulation of BCL6 and c-Myc. The deregulation of hsa-mir-127 in EBV-positive BL may well explain how a post-germinal center cell acquire an immunophenotype of germinal center due to the over expression of hsa-mir-127.

GCET1 EXPRESSION IN ENDEMIC BURKITT'S LYMPHOMA. CORRELATION WITH EBV STATUS AND IGH MUTATION PATTERN

Cristiana Bellan[1], Stefano Lazzi[1], Mario Cocco[1], Teresa Amato[1], *
Giulia De Falco[1], Eleonora Leucci[1], Giuseppina Antonicelli[1],
Nazzareno Palummo[1], Susanna Mannucci[1], Piero Tosi[1], Miguel Piris[2],
Lorenzo Leoncini[1]

[1]Department of Human Pathology and Oncology, University of Siena, Italy;
[2]Lymphoma Group, Spanish National Cancer Centre (CNIO), Madrid, Spain

Gcet1(serpin A9), a gene that belongs to the serpin family of proteins, is located on chromosome 14q32. It is induced when B cells are stimulated with CD40L and it may play an important role in GC-B cell physiology and survival. Previous studies have shown that the expression of GCET1 is primarily restricted to GC B-cells (centroblasts and large centrocytes but not small centrocytes) and also to lymphoid malignancies with GC B-cell maturation. The BL exhibit a heterogeneous staining for GCET1 that probably reflects the eterogeneity of cell of origin of this lymphoma. The normal counterpart of the neoplastic B cells in BL is still unclear. Based on immunoglobulin gene rearrangement studies, some authors suggest an origin from germinal center cells and others from memory B cells. To better clarify the origin of BL we analysed the GCET1 expression on 38 endemic BL, and we correlated the GCET1 expression with the EBV status and with the immunoglobulin mutation pattern of these cases. For that purpose we have performed immunohistochemical analysis using a newly generated monoclonal antibody reactive in paraffin embedded tissues and EBV RNA in situ hybridization (ISH) using. A seminested polymerase chain reaction (PCR) to amplify the VDJ rearrangements of the immunoglobulin heavy chain (*VH*) genes was perfomed and the resultant amplificates were sequenced for comparison with known germline VH segments. We found that GCET1 expression was significantly correlated with EBV status, infact most of the EBV + cases were GCET1-, while most of the EBV- cases were GCET1+ . Interestingly, the mutation pattern of Ig genes differed between EBV-/GCET1+ cases and EBV+/GCET1- cases with an average mutation frequency 1.7 and 5.1 respectively. In addition, we found signs of antigen selection only in EBV+/GCET1 – cases. All toogheter these results again suggested that EBV+ and EBV- BL may originate from distinct subsets of B cells, pointing to a particular role for the germinal center reaction in the pathogenesis of these tumors.

GENOMIC GAIN OF AURORA KINASE A IS FREQUENTLY FOUND IN BURKITT LYMPHOMA AND MAY HAVE SOME EFFECT ON CYCLIN D3 OVEREXPRESSION.

Bibiana I. Ferreira[1], SM Rodriguez-Pinilla[2] , Miguel A. Piris[2],
Juan C. Cigudosa[1]

[1]Molecular Cytogenetics Group and [2]Lymphoma Group. Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid. Spain

Burkitt lymphoma (BL) is one of the most aggressive types of B cell lymphoma. Apart from the pathogenomonic translocation t(8;14)(q24;q32), which results in the rearrangement of the CMYC oncogen, additional or secondary aberrations are frequent in BL that may play a relevant role in the variable clinical outcome of BL patients. Aurora A (AURKA) is a centrosome-associated serine/threonine kinase, which overexpression (normally due to gene amplification) leads to centrosome amplification and chromosomal instability. It has been associated with a worse prognosis in breast cancer patients. A putative similar role has been proposed for this gene in Non-Hodgkin lymphoma but nothing is known in BL.
AIMS: To evaluate the genomic status (number of copies) of AURKA in BL and to place in context this anomaly within the protein expression profile of this type of lymphoma.
M&M: A TMA containing 60 samples of BL (39 cases) and Burkitt-like diffuse large B-cell limphomas (21 cases) was subjected to FISH analysis: AURKA probe was designed to cover the gene locus (RP5-1167H4). A breakapart CMYC probe from DAKO (Cat # Y5410) was used for proper BL diagnosis. FISH assay was carried with standard protocols. Immunohistochemistry was done for: cyclins (A,B,D3,E) and other markers (CD10, CD44, CD23, c-myc) Results and discussion:

1. While 80% of BL showed rearrangement of CMYC by FISH, only 33% of B-like cases were positive.
2. Gains or duplications (3 or 4 copies) of the AURKA gene were observed in 24 cases (53%): 48% in BL and 62% in B-like. These gains consistently appeared in discrete areas of the tumours, suggesting a secondary role. No high level amplifications of AURKA were detected.
3. The protein expression analysis revealed a trend (p=0.066) in the association between cyclin D2/D3 overexpression and AURKA gain. In fact, cyclin D3 overexpression was also found significantly associated with the presence of CMYC rearrangement and an evident cytoplasmic stain for the cmyc antibody.

EPIGENETIC SILENCING OF P16 AND P21 IN GAL-01 SPORADIC BURKITT LYMPHOMA CELL LINE.

Annalisa Roberti[1,2], Giulia De Falco[1,2], Eleonora Leucci[1], Giovanna Cerino[1],
Alessandro Carugi[1], Marcella Macaluso[1], Laurence de Leval[3],
Lorenzo Leoncini[1], Antonio Giordano[1,2]

[1]Sbarro Institute for Cancer Research & Molecular Medicine, Biolife Science Building Suite 333, Temple University Philadelphia, PA19122, USA; [2]Department of Human Pathology and Oncology, University of Siena; [3]Department of Pathology, C.H.U. of Liège, Liege, Belgium.

Burkitt lymphoma (BL) is an aggressive B-cell tumor characterized by high growth rate with a large fraction of cycling cells. All Burkitt lymphomas (BLs) carry reciprocal chromosomal translocations that activate the c-myc oncogene through juxtaposition to one of the immunoglobulin (Ig) loci, providing a constitutive proliferative signal.
This genetic anomaly is the critical event in BL development, but subsequent tumor progression involves the selection of additional genetic and epigenetic changes. It has been reported that both pRb and P53 pathways play a pivotal rule in this process.
The alteration of these pathway is well know in endemic Burkitt lymphomas BLe but no data as reported for sporadic ones. Here we showed that p16 and p21 expression are altered in Gal-01, a new sporadic BL cell line.
Western blotting and immunocytochemistry assay have revealed the lack of p16/INK4a protein in the Gal1 cell line. Methylation-Specific PCR (MSP) revealed that loss of p16/INK4a is due to improper methylation of the promoter region. 5-Aza-2 deoxycytidine (5-Aza-CdR) treatment reactivates p16/INK4a expression and specifically inhibited tumor cell growth, suggesting that aberrant promoter methylation is a common mechanism in sporadic burkitt lymphoma. At the same time, we analyzed the expression changes of some cell cycle regulating genes after demethylating tratment. 5-Aza-CdR treatment significantly increased the level of $p21^{WAF1/CIP1}$ without changing the amount of its direct activator p53. It suggests that also $p21^{WAF1/CIP1}$ may be silenced by aberrant promoter methylation in Gal1 cell line. These data demonstrated at the first time that loss of expression of p21 and p16 by promoter methylation may be a common mechanism in addition with c-myc overexpression in sporadic Burkitt lymphoma progression.

MOLECULAR PROFILING OF BURKITT LYMPHOMA

TRANSCRIPTIONAL GENE EXPRESSION PROFILING OF SPORADIC AND ENDEMIC BURKITT LYMPHOMAS IN RELATION TO OTHER TYPES OF AGGRESSIVE B-CELL LYMPHOMAS

Harald Stein[1], Stefan Bentink[2], Rainer Spang[2], Dido Lenze[1],
Giulia De Falco[3], Cristiana Bellan[3], Mario Cocco[3], Robert Iriso[4],
Lorenzo Leoncini[3], Michael Hummel[1]

[1]Institut für Pathologie, Charité – Berlin, Campus Benjamin Franklin;
[2]Institut für funktionelle Genomik, Computational Diagnostics Group, Regensburg; [3]Department of Human Pathology and Oncology, Division of Pathological Anatomy, University of Siena, Siena, Italy; [4]Lacor Hospital, Gulu, Uganda

The diagnosis of (sporadic) Burkitt lymphoma is not reliabe by means of histology, immunohistochemistry, and FISH. Therefore, we attempted to develop a molecular signature for a more accurate definition of Burkitt lymphoma. Our approaches led to a detection of a signature which appears to be highly specific for Burkitt lymphoma. The application of this signature for the identification of BLs in a large collection of aggressive B-cell lymphomas revealed that the morphological and immunophenotypical spectrum of molecularly defined BL is indeed much broader than previously anticipated. Up to one third of the molecular BLs proved to have the morphology of diffuse large B-cell lymphomas and up to 20% of them expressed BCL-2. Even the MYC-break was missing in 10% of the cases. The specificity of our signature was confirmed by a noval independent bioinformatic approach established very recently in our group. The specificity of our molecular Burkitt signature

was additionally confirmed by applying it to the lymphoma collection of aggressive B-cell lymphomas described by Dave et al (NEJM June 2006). This led to the identification of the same cases which Dave et al had identified as Burkitt lymphomas by using a totally different gene expression signature. With information in mind we have now applied our gene expression signature to endemic Burkitt lymphoma. The results obtained are compared with those found for sporadic Burkitt lymphoma.

Micro RNA EXPRESSION PROFILING OF MATURE AGGRESSIVE B-CELL LYMPHOMA WITH SPECIAL REFERENCE TO SPORADIC AND ENDEMIC BURKITT LYMPHOMA

Dido Lenze[1], Michael Hummel[1], Korinna Jöhrens[1],Eleonora Leucci[2], Anna Onnis[2], Lorenzo Leoncini[2] and Harald Stein[1]

[1]Institute of Pathology, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin; Germany
[2]Dipartimento di Patologia Umana e Oncologia, Università degli Studi di Siena, Italy

A delineation of diffuse large B-cell lymphoma (DLBCL) from Burkitt lymphoma (BL) or a distinction of clinical relevant subgroups within DLBCL is not reliably possible by means of histology, immunhistochemistry and FISH analysis (MYC break). Therefore gene expression profiling has been performed in order to identify molecular subgroups which are not dependent on morphological or immunophenotypic criteria. This led to the discovery of a gene signature which is able to identify BL cases among other aggressive B-cell lymphomas and divide DLBCL into cases derived from activated B-cells (ABC) and germinal centre B-cells (GCB), respectively. However, both molecular classifications leave a significant proportion of cases unclassified, demonstrating a need for alternative molecular classifications. Since the analysis of microRNA expression is described as an additional approach for the identifications of molecular and clinical relevant subgroups within disease entities, we extracted RNA from 30 eBL and sBL cases as well as from 288 DLBCL cases treated with CHOP with or without addition of Rituximab. The analysis of the microRNA expression was performed with microarrays carrying 303 human mircoRNAs and subsequent bioinformatic interpretations (ANOVA, Partitioning clustering) were carried out in order to detect differentially expressed microRNAs. 81 microRNAs were found to differ in their expression between histologically defined DLBCL and BL whereas less microRNAs were able to distinguish between ABC- and GCB-type of DLBCL. The BL cases as defined by their microRNA expression profile comprise not only cases with a morphology of BL but also cases with a DLBCL morphology. In addition, the microRNAs were able to subdivide the remaining cases lacking the microRNA BL signature into groups with clinical relevance. Our data show that the expression differences of microRNAs provide a valuable tool for the distinguishing of BL from DLBCL and adds to the dissection of DLBCL into prognostic molecular subgroups. Furthermore, some microRNAs are differentially expressed between sBL and eBL supporting the notion that they do not represent completely identical disease entities.

RBL2 NETWORK IN ENDEMIC BURKITT LYMPHOMA EXPLORED BY GENE EXPRESSION ANALYSIS.

Pier Paolo Piccaluga[1], Giulia De Falco[2,3], Andrea Califano[4], Eleonora Leucci[2], Annalisa Roberti[2,4], Robert Lukande[5], Lynette Tumuwine[5], Robert Iriso[6], Chiara Lucchetti[2,4], Antonio Giordano[2,3], Stefano A Pileri[1] and Lorenzo Leoncini[2]

[1]Unit of Hematopathology, Institute of Hematology and Medical Oncology L. and A. Seŕgnoli, S. Orsola-Malpighi Hospital, University of Bologna, Italy. [2]Dept. of Human Pathology and Oncology, University of Siena, Italy. [3]College of Science & Technology, Sbarro Institute for Cancer Research & Molecular Medicine, Temple University, Philadelphia PA 19122, USA. [4]Center for Computational Biology and Biochemistry, Columbia University, 10032 New York, NY, USA. [5]Department of Pathology, Makerere University Medical School, P.O. Box 7072, Kampala, Uganda, [6]Lacor Hospital, Gulu, Uganda

Burkitt lymphoma (BL), the most common lymphoma type in Africa, is a B-cell tumor typically characterized by translocation t(8;14)(q24;q32), leading to MYC over-expression. However, several additional alterations have also been described in BL, including RBL2 gene mutations. RBL2 belongs to the retinoblastoma family which is composed by three members, pRB/p105, p107 and RBL2/p130. Though through different mechanisms, they regulate cell cycle progression by inducing cell growth inhibition and arrest in different cell lines. It has been previously demonstrated that RBL2 is commonly mutated in endemic BL, with consequent nuclear localization and functional inactivation of the encoded protein, pRb2/p130. This raises the intriguing possibility that RBL2 pathway alteration might play a role in BL pathogenesis. Recently, we showed that RBL2/p130 function restoration in BL cell lines determined regain of growth control, through the regulation of several genes.
We thus performed a gene expression profile (GEP) analysis of endemic BL cell lines, transfected with either wild type RBL2 or empty vector, and primary cases and normal B-cell subpopulations in order to 1) assess whether RBL2 dependent pathway is actually shut off in endemic BL; 2) assess whether introduction of functional RBL2 in BL cells could restore a functional pathway consistent with that of normal B-cells; 3) identify possible RBL2 target genes. ŕňmvcxound that RBL2 -transfected cells had a global GEP relatively similar to that of wild type cells. However, we could identify a RBL2 dependent molecular signature which could clearly distinguish the samples according to RBL2 mutational status. Among others, this signature included genes relevant for cell cycle regulation. Analysis on primary cases and normal B-cells is ongoing at present. In addition, identification and characterization of RBL2 target genes is under evaluation by using the ARACNE algorithm. Results will be presented and discussed at the meeting.

INDUCTION OF A HODGKIN-LIKE PHENOTYPE OF BURKITT LYMPHOMA CELLS BY EPIGENETIC REPROGRAMMING

Michael Hummel[1], Anke Ehlers[1], Elisabeth Oker[1], Stefan Bentink[1], Dido Lenze[1], Harald Stein[1]

[1]Institut für Pathologie, Charité – Berlin, Campus Benjamin Franklin; [2]Institut für funktionelle Genomik, Computational Diagnostics Group, Regensburg

The tumor cells of Burkitt lymphoma (BL) are regarded as derived from early germinal centre B-cells. They consistently show an immunophenotype of mature B-cells with expression of all B-cell typical antigens and transcription factors. In contrast, the tumor cells of classic Hodgkin lymphoma (HRS cells), which are also thought to derive from germinal centre B-cells, have almost completely lost their B-cell identity. In addition, HRS cells have acquired the expression of several antigens which are regarded as B-cell lineage inappropriate. We and others suggested that epigenetic events are involved in the silencing of B-cell associated genes as well as in the upregulation of B-cell inappropriate genes. In order to determine this possibility we treated both Hodgkin and BL cell lines with DNA-demethylating (5-aza-dC) and histone-acetylating (TSA) reagents. Treated and untreated cell lines were analysed by Affymetrix GeneChips. Numerous up- and down regulated genes were found. This was verified by quantitative RT-PCR and Western blot analysis. Chromatin-immunoprecipitation was carried out to determine the epigenetic modifications in the promoter region of the corresponding genes. Against all expectations the treatment of Hodgkin cell lines with demethylating and acetylating reagents did not restore the B-cell expression program or parts of it. Instead, the treatment of BL cells resulted in a complete loss of their B-cell phenotype and – in parallel – to an up-regulation of Hodgkin-characteristic genes. Our data clearly demonstrate that DNA-demethylation and histone-acetylation is able to re-program BL cells into cells with a Hodgkin-like phenotype. These findings imply that demethylation and acetylation up-regulate genes in BL cells that down-regulate the genes responsible for the B-cell expression program. These findings suggest that the same genes which are switched on in BL cells by DNA-demethylation and histone-acetylation are constitutively active in Hodgkin cells. It is tempting to speculate that these genes are not only involved in the extinction of the B-cell phenotype but also in the pathogenesis of Hodgkin lymphoma.

CLINICAL ASPECTS, CHEMOTERAPY AND NOVEL THERAPEUTIC TARGETS IN BURKITT LYMPHOMA

NON-ENDEMIC BURKITT'S LYMPHOMA IN MULAGO HOSPITAL, KAMPALA.

Dr. Birabwa-Male Doreen, Dr. Sekabira John, Dr. Namuguzi Daniel

Makerere University, Kampala, Uganda

Denis Burkitt, while working in West Nile, Uganda, discovered a jaw tumour in Children which was later called Burkitt's lymphoma (BL). The same tumour was later found to affect other abdominal and thoracic organs. The Former is unique to Sub-saharan Africa while the latter can be found else-

where in the world as well. The pattern in Uganda was looked at as it occurs in Mulago Hospital (MH).
Methods: In Uganda most of these patients before getting chemotherapy pass through the Paediatric Surgical Unit (PSU) of MH for diagnosis. Hospital records of these patients from 2005 – 2007 were looked at and analysed. The author has been looking after these children since.
Results: There were 73 pts with non-endemic BL seen at the PSU. Their age ranged from 2yrs – 12yrs. There were 30 males and 43 females. The most affected organ was the ovary, 59% and there was only one testicular involvement. The other affected organs included liver, kidney and aortic lymph nodes. Most of these patients come late on average > 6/12.
Conclusion: Non- endemic BL is a very common tumour seen in Uganda. More efforts should be applied to pick it up early for better results.

SURPRISING PROFILE OF INCIDENT CASES OF BURKITT S LYMPHOMA IN THE LAKE ZONE IN TANZANIA.

Schmidt C, Kayange N, Festo E, Kahima, Bayange M

Department of Paediatrics, Bugando University College of Health Sciences (BUCHS), Mwanza, Tanzania

Background: Burkitt`s Lymphoma (BL) is the most important malignant disease of childhood in East Africa. We describe the profile of recent incident cases and the current approaches to diagnosis and therapy in our Department at the Bugando Medical Centre (BMC) in the Lake Zone, Tanzania.
Methods: In a period of six months (April-Sept. 2007) we admitted more than 60 children with a diagnosis of BL. Children with suspected BL underwent fine needle aspiration (FNA), if necessary and in all cases of abdominal mass under ultrasound (US) control, bone marrow aspiration (BMA) and lumbar puncture for assessment of cerebrospinal fluid (CSF) were performed. Full blood picture (FBP), ELISA for HIV, liver and renal function tests (LFT and RFT), and abdominal US were done.
Results: The diagnosis of BL was confirmed in 52 patients. 26 (50%) children presented with abdominal mass, 22 (42%) head and 4 presented with combinations of abdominal mass and jaws. In total in our collective we found 30 (58%) children with an intraabdominal mass. The median age was 6.2 years. 35 (67%) children were male, 17 (33%) female. Only 2 (4%) patients out of the total number were HIV positive.
Discussion: The African type of BL is characterized by a predominant cephalic location. Although it is known that clinical features varied significantly by different regions in East Africa, the high number of abdominal BL in our children is remarkable. On the other hand the very low prevalence of HIV in our children with BL is surprising. The prevalence of HIV in our children with BL was even lower than the prevalence of all children of the Lake Zone in Tanzania.
Conclusions: A remarkable incidence profile was found which demands further studies in addition to the outstanding efficacy investigations. The new approach to BL at BMC/BUCHS has facilitated more appropriate treatment of children with BL.

TRENDS ON CHEMOTHERAPY IN BURKITT LYMPHOMA

C Patte

Institut Gustave Roussy, Villejuif, France.

Burkitt lymphoma (BL) is the most frequent lymphoma in children, with a higher frequency in the so-called “endemic area”. Abdomen and face are the most frequent tumor sites. It generally presents as advanced stage disease. Bone marrow and CNS is involved in about 20% of the cases. Its outcome greatly improved due to several prospective (multi)national studies.
Treatment must be done by intensive pulse polychemotherapy courses, but of short duration, relapses occurring early, within the first year. The French LMB studies told us that :a) HDMTX is a very effective drug for prevention of CNS disease b) LAL-B and CNS positive patients benefited from the increase of the dose of HD MTX and the introduction of HD ARA-C (CYVE courses), c) treatment intensity can be adapted to stage and resection, but also to response to chemotherapy (at D7 and after 3 courses), d) early dose intensity is essential. The German BFM studies told us that a) treatment intensity can be adapted to stage and resection, but also to LDH level, b)HDMTX is a very effective systemic drug, and exposition to the drug must be all the more long as the disease is more advanced. They also confirmed that HD Ara-C is important in the advanced diseases. Other studies showed that treatment duration can be of short duration and confirmed that cyclophosphamide, HDMTX and Ara-c are the major drugs in BL, in addition to vincristine, doxorubicin, VP16, corticosteroids. CNS prophylaxis must be done by HDMTX +/- HD Ara-C and IT injections of MTX +/-Ara-C, but not by cranial irradiation. With all these current strategies in Western countries, EFS of BL increase to 80-90%, but toxicity of the treatment is high and needs adequate supportive care. CNS disease remains a bad prognostic factor, with EFS <80%. Next questions concern the use of targeted therapy (rituximab), and the modalities of improving outcome of poor risk patients: those with CNS disease, with poor initial responses, and those who relapsed. In less privileged countries, where BL is frequent, EFS might not be so high, mainly due to later diagnosis with children arriving in very bad general condition and to lesser access to drugs and to supportive care. Cyclophosphamide alone with ITMTX might cure 30-50% of the endemic cases with less cost.
In conclusion, story of Burkitt lymphoma was a successful story especially in “privileged” countries: where EFS increased from 30-50% to 80-90% in 20 years, while weight of treatment decreased for the majority of the patients. This must encourage the cooperation between countries to aim to cure as many patients in “less privileged” countries.

BURKITT’S LYMPHOMA TREATMENT AT THE KENYATTA NATIONAL HOSPITAL.

Mwanda O.W

University of Nairobi, Kenya

Background. Treatment of BL is not well endowed settings remain challenging. The usual approach is to tailor the treatment to prognostic factors, feasibility of the drug schedule, drug management and the capacity of the setting. Immediate, intermediate and long term management. Methods from 2001 to 2005 we studied patients age 3 to 10 years who were treated for stage B C and D BL. We compared four cycles of Vincristine, cyclphosphamide, prednisone and adrimycin (CHOP), methotraxate, cyclophophamide, vincristine, prednisone and reviewed literature for other drug combinations.
Results: The median follow-up was 60 months. The estimated two year event free survival rate was significantly higher after CHOP compared to methotraxate based and other drug schedules. The drug management was also best in CHOP compared to methotraxate based drugs most of the literature protocols were not found feasible in our settings.
Conclusion: Cytotoxic drugs improve the lives of many cases of BL, standard treatment remains CHOP/CHOP-M is the most feasible in our setting.

MULTI-CENTER STUDY OF THE TREATMENT AND CHARACTERIZATION OF BURKITT LYMPHOMA (BL) IN AFRICA

Ngoma T[1], Kaijage J[1], Githang’a J[2], Durosinmi MA[3], Adeodu OO[3], Salawu L[3], Aken’Ova YA[4], Brown BJ[4], Rohatiner AZ[5], Venzon D[6], Adde M[5], Magrath I[5]

[1]Ocean Road Cancer Institute, Dar es Salaam, Tanzania [2]Kenyatta National Hospital, Nairobi, Kenya [3]Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria [4]University College Hospital, Ibadan, Nigeria [5]International Network for Cancer Treatment and Research, Brussels, Belgium [6]Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

Purpose: The objectives of this study were to characterize the presentation features of BL and to assess the response, event-free survival (EFS) and overall survival (OS) in patients treated according to a uniform protocol and to assess efficacy of a salvage regimen. It was conducted by 4 institutions in three African countries, Nigeria, Kenya and Tanzania.
Patients and Methods: All previously untreated patients with BL were eligible for the study, including those with bone marrow involvement and/or CNS disease. Treatment consisted of First-Line (FL) regimen for newly diagnosed patients and a Second-Line (SL) regimen for patients who failed to respond to or who relapsed after FL. FL consisted of 6 cycles of cyclophosphamide, vincristine, methotrexate (MTX) plus IT therapy with both MTX and ara-C. SL therapy consisted of 4 cycles of ifosfamide with mesna, etoposide, ara-C plus IT MTX and IT ara-C.
Results: A total of 205 patients (137 males and 68 females) were entered on the study. The median age was 7 years (range, 7 months to 28 years). The most common presentation features were jaw tumors (61%) followed by abdominal disease (57%). To date, 142 patients (73%) have achieved CR, 31 (16%), PR, and 5 (2%), NR to FL therapy. Seventeen patients (8%) could not be evaluated for response (death during induction or failure to return for evaluation). Ten are too early in FL to assess response. Twenty patients relapsed. A total of 33

patients have received SL therapy; 10 relapses and 23 PR/NR to FL therapy. Twelve patients achieved a CR to SL and 18 a PR or NR. Eleven of these 12 CRs remain alive and free of disease. Survival analyses were performing using the Kaplan-Meier method. EFS is 53% at 12 months and 50% at 24 months. OS is estimated at 69% at 12 months and 63% at 24 months.
Conclusions: This regimen is feasible in African countries and the results to date are encouraging. Patients who relapse or who fail to respond can be salvaged with SL treatment.

THE TREATMENT OF CHILDHOOD'S BURKITT'S LYMPHOMA IN AFRICA BY THE FRENCH-AFRICAN PEDIATRIC ONCOLOGY GROUP (G.F.A.O.P.)* A STUDY OF 714 CASES REGISTERED FROM APRIL 2001 TO AUGUST 2007

J.Lemerle, M.Harif, M.Khattab, F.M.Alaoui, P.Doumbe; S.Barsaoui,Y. Ladjadj, N.Cherif, C.Moreira, B.Togo, F.Traore; J.Andoh, Couitchere, JJ.Atteby, L.Kam, Ye,F.Rafaramino, H.Raobijaona, N.Ravelomanana, A.Auperin, M.A.Raquin, ,M.Raphael, C.Patte

*C /O Institut de Cancerologie Gustave Roussy, 94805 Villejuif , France.

1) GFAOP performed a first prospective multicentric study in 2001-2004, aiming at assessing the feasibility of adapted French protocols in Northern, Western, and Central Africa, and Madagascar.
From 04 01 to 04 04, 343 patients were referred to 9 Units in 6 African countries,306 were suitable for study.
The 2 tested schemes derive from LMB 89 Protocol. One, MAT, is very similar to the model, and was used in the North. The other, LB 2001, less aggressive, was used in the South. Both contain CPM,VCR, PN, ARA C, MTX 3g repeated courses, and I.T. therapy. Neither surgery nore XRT. Slides were centrally reviewed.
Patients. Stages were 1- 2: 14%, 3 : 7O% , 4: 16%. Tumor sites: Abdomen in 242; Face and/or jaw in 131. Most patients had advanced disease and miserable general condition, malnutrition in 39%, anemia, fever, infection.
Treatment. 187 PTS received LB 2001 ,and 119 MAT. .
Results. Three yr. O.S. is 61 %,higher in the MAT Group. 76 pts. died during treatment, 59 during induction ,. Relapses occurred later. with LB.
Treatment –related mortality declined from 27% to 10%during the first 3yrs., the overall survival raising simultaneously from 54 to 73% .
Conclusion: efficient intensive multidrug chemotherapy can be used successfully in the mentioned areas of Africa . Toxicity however is a problem.
2) Second GFAOP studies 2005-2007, ongoing in 11 Units, in 9 countries. The MAT protocol is being continued unchanged in 5 Units in Morocco, Algeria, Tunisia. The results are stable on the first 155 pts. A new study of Cyclophosphamide. plus I.T MTX started in 04 05, as an attempt to reproduce and to improve the results of P.Hesseling et al. with CPM alone , by adding an early"rescue"scheme ,in cases of poor response to CPM. 216 cases registered and 148 included 04 05 to 10 07.in 6 Units south to Sahara. Limited toxicity. Overall survival 50%. Unproven efficiency of the "rescue"scheme.
Conclusion: accurate analyses, by Unit, and longer FU needed.

THE INTENSIVE SHORT-TERM CHEMOTHERAPY REGIMEN DESIGNED AT INT MILAN IS HIGHLY EFFECTIVE FOR THE TREATMENT OF BOTH CHILDREN AND ADULTS WITH BURKITT S LYMPHOMA.

E. Schiavello[1], F. Spreafico[2], A. Guidetti[1], M. Massimino[2], R. Luksch[2], F. Fossati-Bellani[2], A.M. Gianni[1], and M. Di Nicola[1]

C. Gandini Medical Oncology, Bone Marrow Transplantation Unit[1] and Pediatric Oncology Unit[2], Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

Background. We previously showed the outcome of Burkitt s lymphoma (BL) achieved by a short intensive sequential chemotherapy program initially scheduled for pediatric population and also used for adults in our Institute . Purpose. We have analyzed the clinical outcome of 69 newly diagnosed BL patients(pts), aged between 2 and 76 years (median 11 years) homogeneously treated with the chemotherapy regimen originally designed for children to evaluate differences based on age and prognostic factors (stage IV Murphy and/or CNS involvement).
Methods. Main patient characteristics were: male/female 51/18; age below 18 years in 46 cases, ≤33/≥33 years 56/13, stage I-III/IV 51/18. Seven out of 18 stage IV pts exhibited CNS involvement. Sequential chemotherapy included: after a 5-week induction phase of weekly infusion consisting of vincristine, cyclophosphamide, doxorubicin, HD-methotrexate plus leukovorin rescue, and intrathecal methotrexate or AraC, a consolidation phase including HD-AraC plus cisplatin was given.
Results. Overall 9/69 pts suffered from disease failure after median 5 months from diagnosis (range, 2-31 months). Two toxic deaths occurred. EFS, DFS, OS at 3 years were 83.5% ± 5%, 86% ± 4%, and 87% ± 4% respectively, for 69 evaluable pts. No significant differences in EFS, DFS and OS were observed between pts ≤33 years vs. ≥33 years In addition, also for pts with stage IV vs. stage I-II-III no significant differences in OS and DFS were observed. Pts with CNS involvement showed a lower OS and DFS compared with no CNS involvement however without reaching statistical significance.
Conclusion. Our data strongly suggest that the intensive pediatric chemotherapy regimen demonstrated to be very effective and feasible in both pediatric and adult pts. Moreover, we confirm the efficacy also in poor prognosis pts stage IV, especially CNS-positive. The addition of Rituximab to chemotherapy regimen in a subset of pts is under clinical investigation.

THE SYMPTOM BURDEN AT PRESENTATION AND AFTER 3 CYCLES OF CHEMOTHERAPY IN CHILDREN WITH BURKITT'S LYMPHOMA AT MULAGO HOSPITAL, KAMPALA, UGANDA.

Katie Wakeham[1,2], Jonathan Levin[1] Caroline Rose[2]

[1]Medical Research Council and Uganda Virus Research Institute, [2]Hospice Africa, Uganda

Background: There is no published data on the presenting symptoms of children in Uganda with BL, or on the side effects associated with treatment. Given the lack of research on this important issue, we aim to generate evidence regarding the symptom burden of Ugandan children at presentation and after 3 cycles of chemotherapy, in order to inform clinical practice and policy.
Methods: Hospice Africa, Uganda (HAU) provides symptom management and chemotherapy to children presenting with BL at Mulago Hospital. A retrospective analysis of HAU records (n=96) was carried out. Presenting symptoms and symptoms present between cycles 3 and 4 of chemotherapy were recorded for biopsy confirmed children with BL. All children were presenting with BL for the first time.
Results: 54 children were HIV negative; 4 were HIV positive and 38 had unknown status. The children's ages ranged from 2 to 16, with a median age of 6.5 (IQR 5-9). The major occupation for parents was subsistence farming (n>80, 83%),and no family was deemed able to pay for their child's chemotherapy. There were slightly more boys than girls (53% vs 47%). All children presented at an advanced stage of BL. The most common sites of disease were the jaw and abdomen. The most common presenting symptom was a mass (97%), followed by pain (82%), low mood (42%), sore mouth (29%), fatigue (28%), halitosis (23%), cachexia (23%) and loss of appetite (22%). 10 children presented with spinal cord compression (10%) and 5 with blindness (5%). After 3 cycles of chemotherapy the most common symptom was a mass (48%), pain (43%), loss of appetite (29%), nausea (25%), clinical evidence of infection (23%), cough (21%), vomiting (21%) and a fever (13%). 6 children died and 10 children were lost to follow up in the period between chemotherapy cycles 1 and 3.
Conclusion: This study shows that children with BL in Uganda present with advanced stage disease, most commonly with a mass and pain. The majority of parents are subsistence farmers, suggesting that finances, health awareness and access are likely to be major issues surrounding these advanced presentations. After 3 cycles of chemotherapy the prevalence of a mass and pain reduces, but these remain the most common symptoms. At this stage loss of appetite, nausea and vomiting become important symptoms to be addressed. On the basis of our findings, we make the following recommendations:
- Rigorous research to assess the morbidity associated with chemotherapy for BL
- Public education and health worker training to improve awareness of BL and promote early referral and access to treatment.
- Development and promotion of a co-ordinated African BL programme.

A COMPARISON OF OUTCOME AND TOXICITY OF SHORT AND LONG COURSE CHEMOTHERAPY FOR BURKITT'S LYMPHOMA (BL) AT KENYATTA NATIONAL HOSPITAL (KNH)

William M Macharia

Department of Paediatrics & Child Health, AKU, Nairobi, Kenya

Objective: To compare the effectiveness and toxicity of an intensive, short course (SC) and a less intensive long course (LC) chemotherapy regimen in treatment of BL.

Study Design: Retrospective Cohort
Methods: The study was undertaken at KNH using patients' records. Children 15 years and below admitted in general wards or in paediatric oncology ward with fine needle aspirate or histological diagnosis of BL were eligible for inclusion. Death before completion of induction and exposure to other protocols prompted exclusion.
Analysis: Median duration of follow-up, event free survival (EFS) and treatment related toxicity.
Results: Of 101 records, only 25.7% were considered suitable for analysis. There were more deaths before completion of induction in the general children wards (77.8%) than in the paediatric oncology ward (22.2%). Diagnosis was confirmed by FNA in 78% and histology in 47.3% of the cases. 50% f patients had abnormal CSF cytology at presentation. There was no significant difference in EFS and toxicity between the two treatment groups. 19.2% received packed cells and 3.8% platelet concentrates while 53.8% experienced moderate to severe neutropenia. 53.8% required i.v. antibiotics (58.3% in SC and 50% in LC group). There was not significant difference in toxicity between the two groups.
Conclusion: Outcome of BL treatment remains poor with many patients dying prior to commencement of chemotherapy. In the absence of clear difference in effectiveness and toxicity between SC and LC protocols, choice should be based on treatment cost and informed parental choice. Early commencement of therapy and improvement in supportive care may lead to better outcomes. Prospective data is recommended for more valid deductions from clinical experience.

A NEW SHORT-TERM HIGH INTENSIVE PROTOCOL BL-M-04 FOR ADULT PATIENTS WITH BURKITT LYMPHOMA: EFFICACY AND TOXICITY.

Baryakh E, Zvonkov E, Kremenetskaja A, Kravchenco S, Magomedova A, Obukhova T, Klyasova G, Yatskov K., Valiev T, Vorobjov A

Russian Hematological Scientific Center, Russian Academy of Medical Science, Moscow, Russian Federation

Aim: to evaluate an efficacy and toxicity of therapy protocol BL-M-04 for adult patients with Burkitt lymphoma (BL).
Patients and methods: twenty five previously untreated patients with BL (they had specific for BL cytogenetic aberration t(8;14)(q24;q32)) or variant translocation t(2;8), t(8;22) were eligible for inclusion in our study. All the patients (17 males and 8 females, mean age 24 years (from 15 to 56 years)) participated in the study performed in Russian Hematological Research Center between August 2003 and September 2007. A treatment was based on experimental high intensive protocol BL-M-04. The BL staging criteria developed by S. B. Murphy was used to stage the patients. The stage I, II, III, IV was diagnosed in 1, 2, 14 and 2 patients respectively. B-acute lymphoblastic leukemia (L3) – in 6 patients. B-symptoms (night sweets, fever, weight loss) showed in 21(84%) patients. Serum lactate dehydrogenase level (LDH) was increased in 22 (88%) patients. The main aim of a new treatment regimen became an intensification and treatment duration reduction in patients with BL. Our new treatment protocol is based on standard NHL-BFM-90 protocol for group of high risk patients (methotrexate dose 1500mg/m2). We know that BL is a chemosensitive tumor and regresses after 1-2 courses of chemotherapy. Despite the initial tumor mass we decided to treat BL according to 4 courses (2 inductional and 2 consolidational). According to the fact that BL is the most sensitive to high dosed methotrexate and cytarabine we used these drugs in the induction phase to achieve the most cytoreductive effect. Courses A and C were used for remission achievement. Doxorubicine was added to course A, methotrexate – to course C. Consolidative courses were the same as inductional courses. So, we used A and C courses (without B), intensified with course B drugs (doxorubicin and methotrexate), interval between courses was 21 day.
Results: twenty two patients (88%) achieved a common remission (CR) after 1-2 courses (11 patients – after the 1st course, 11 – after the 2d). Four patients died. The course of death was chemotherapy complications in three cases, early relapse in one patient. A 3-year disease-free survival – 95%, overall survival – 84%. BL-M-04 therapy is associated with higher CR rates and longer disease-free survival in adult patients with BL and demonstrated a high efficacy of short-term intensive therapy. Treatment duration was 3–3,5 months. Myelotoxic agranulocytosis completed all courses. Most infectious and hemorrhagic complications of treatment were registered during the first course A, that can be explained by initial severe patient condition in the most patients. Unfortunate prognostic factors, which increase the number of chemotherapy complications, are: B-ALL, acute renal failure, inadequate previous treatment (surgery and chemotherapy).
Conclusion: BL-M-04 is a highly effective protocol: a 3-year disease-free survival – 95%, overall survival during 3 years – 84%. This protocol can help us achieve a rapid tumor mass regression and treatment duration reduction, because of chemotherapy intensification and acceptable toxicity. We conclude, that the majority of relapses occur after 8-12 months of treatment and after 24 months we can speak about full recovery. The usage of this protocol can achieve a rapid BL regression and decrease of treatment duration because of treatment intensification and acceptable toxicity.

FISTULAE COMPLICATING BURKITT'S LYMPHOMA THERAPY, THE CHALLENGES: 2 CASE REPORTS.

Kitonyi G.W. (MBchB FRCPath.)

Department of Human Pathology/ Haematology and Blood Transfusion University of Nairobi, School of Medicine, Kenya

A 5 year old boy with a large right maxillary jaw Burkitt's, involving the nose and protruding into the oral cavity developed a 2 cm diameter oral antral fistula, 2 months into treatment with the CHOP regime. The development was dramatic with a choking cough and voice change. Reluctant to interrupt chemotherapy, maxillofacial surgeons fabricated a well fitting orbturator prosthesis, which was misplaced by the patient within 48 hours. Because of the considered risk of choking, chemotherapy was interrupted to allow for corrective surgery. A second patient, 10 year old female, diagnosed with Burkitt's, presented with extensive disease, involving the left jaw, abdomen, central nervous system and bone marrow. Two and a half months after commencement of the CHOP regime, she developed a vesico-vaginal fistula, (VVF). Despite the discomfort the patient was experiencing, surgery was postponed in favour of chemotherapy, in view of the still heavy residual disease burden. The development of fistulae in children with Burkitt's lymphoma, following tumour lysis by chemotherapy, maybe life threatening and incapacitating. It also poses challenges and dilemmas in surgical intervention decisions. This report highlights some of these issues.

CLINICAL PRESENTATION AND DIAGNOSTIC FINDINGS IN BURKITT LYMPHOMAS AT A SINGLE INSTITUTION IN SWEDEN.

Daniel Tesfa[1], Monika Jansson[1], Birgitta Sander[2], Birger Christensson[2], Mats Merup[1]

[1]Hematology Center, [2]Department of Laboratory Medicine, Divison of Pathology, Karolinska University Hospital at Huddinge, Stockholm, Sweden.

Burkitt lymphomas (BL) are rare in Sweden and constitute less than 1% of lymphomas in the Swedish registry. Identification of BL among highly proliferative non-Hodgkin lymphomas (NHL) is important since BL respond poorly to standard therapy for diffuse large B-cell lymphomas (DLBCL). We have analysed 27 adult patients with NHL with a proliferation rate of more than 90% diagnosed at Karolinska University Hospital at Huddinge between 1996 and 2005. Twenty patients were diagnosed as BL and 7 as DLBCL. Average age was 46 for BL (26-83 ys) and 55 (17-88 ys) for DLBCL. Extranodal presentation was seen in 70% of BL compared to 57% for DLBCL and abdominal presentation was seen in 50% of BL patients. Four cases, all BL, had CNS involvement. Elevation of lactade dehydrogenase was seen in 80% of BL compared to 43% of DLBCL and increase of uric acid was found in 50% and 29% respectively The typical immunohistochemical phenotype for BL was expression of CD20 (100%), CD10 (89%), bcl-6 (83%). Expression of bcl-2 was seen in 44% and p53 expression in 25%. All DLBCL expressed bcl-2 and bcl-6 was expressed in 71% and CD10 in 43%. No cases expressed p53. EBER antigen was evaluable in 24 of 27 cases and expression was seen only in three cases, all BL. MYC gene rearrangement was analysed with fluorescence in situ hybridization (FISH) on paraffin sections using two different probes, one for detection of break within the myc gene and one for detection of the t(8;14) translocation. Evidence for myc gene rearrangement was found in 75% of BL and 14% of DLBCL. Twelve of 20 BL patients were treated with the Riehm protocol for Burkitt lymphoma. Five cases received rituximab. Eleven patients obtained complete remission and 8 patients (67%) remained in CR for a median follow up of 24 months. Disease free survival at 2 years was 50% for BL and 33% for DLBCL. We conclude that analysis of myc gene rearrangement with FISH is feasible on paraffin sections and facilitates making a correct diagnosis of BL.

CYTOGENETICS IN BURKITT LYMPHOMA THE IMPACT OF AGE AND GENDER.

EG Boerma[1], R Siebert[2], PhM Kluin[1], M Baudis[3]

[1]Department of Pathology, University Medical Center Groningen, Groningen, The Netherlands, [2]Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany, [3]Institute of Molecular Biology, University of Zurich, Zurich, Switzerland

Since the discovery of Burkitt lymphoma (BL) in 1958 by Denis Burkitt, many investigators have studied its genetic features. Apart from the hallmark MYC-translocation, many chromosomal aberrations have been reported. We used the online Mitelman s Catalog of Chromosome Aberrations in Cancer to determine a cytogenetic profile of BL.
We collected karyotyping data of all 6359 B-NHL. Karyotypes were converted to an 862 band specific status map with breakpoint and imbalance information using the Progenetix software. We selected all cases with a diagnosis of Burkitt lymphoma/leukemia and a MYC-Ig translocation (n=514). Original publications were searched for clinical data. Cases were separated into different age groups (0-15, 16-30, 31-45, 46-60 and >60). Apart from the typical t(8;14) translocation in 417 cases (81%) and variant translocations t(2;8) in 25 (5%) and t(8;22) in 72 cases (14%), we identified 61 double hit cases with an additional structural aberration (gain or translocation) suggestive for 3q27 (BCL6), 11q13 (CyclinD1) or 18q21 (BCL2). The incidence of these double hit cases increased from 6/202 (3%) between 0-15 to 18/45 (40%) >60 years. After exclusion of these cases, 245 of the 453 cases (54%) showed chromosomal imbalances. The most common gains were found at 1q2 (20%), 7q3 (6.2%) and 12q2 (5.1%), most common losses at 13q3 (6.8%), 6q2 and 17p1 (4% each). The average number of chromosomes with imbalances steadily increased with age (from 0.85 to 1.8) and was higher in females than in males (1.22 vs 0.98). Gain of 1q was more common at young age, gain of chr12 at intermediate age and gain of chr7 at high age. Overall, 87% of the cases had 0-2 chromosomal imbalances (0: 46%, 1: 28%, 2: 12%, >2: 13%). The classic cytogenetic profile of BL is a MYC-Ig translocation accompanied with no more than 2 additional aberrations and without a so-called double hit. Even within this genetic homogenous disease there are differences related to age and gender.

IMPLEMENTATION OF A CHILDREN'S PALLIATIVE CARE SERVICE IN A LYMPHOMA TREATMENT CENTRE IN UGANDA

Caroline Rose, Charles Byarugaba, Justin Amery

Hospice Africa Uganda, Kampala, Uganda

This paper will describe the planning and implementation of a children's palliative care service in Uganda at Lymphoma Treatment Centre, Mulago Hospital. This programme began in January 2007 with the recruitment of a children's services coordinator and continues to the present time. Children's palliative care in Africa is a much needed but extremely new field.
The paper will describe the six main strands of the project.

1. Initial situation analysis and planning
2. Recruitment of a Ugandan Children's Services Coordinator (supported by an international volunteer from a children's palliative care and oncology nursing background)
3. Provision of symptom care from diagnosis to end of life for children diagnosed with life limiting illness
4. The implementation of a hospital based volunteer led advocacy, play and education service
5. Production of IEC materials to improve family and child education about their illness and prognosis.
6. Monitoring and Evaluation of the service

The paper will discuss the challenges faced by the team which included resistance to the development of the project, firmly held myths about children's pain and pain management and a lack of available resources. It will also discuss the steps taken to overcome these challenges.
Early recommendations coming from this project include the need to ensure that both pharmacological and non-pharmacological treatments are used to treat pain and intervention pain in children. The need for and interest in specialist training of palliative care staff in paediatric palliative care should also be recognised. Finally it is recommended that facilities providing palliative care should develop written and pictorial tools in order that families have the information about their child's illness and prognosis to enable them to make informed decisions and choices about their child's care.

BURKITT'S LYMPHOMA AND ANGIOCENTRIC NK/T CELL LYMPHOMA; PERSONAL EXPERIENCE N THE LAST 45 YEARS IN SUDAN

A M EL Hassan DSs, FRCP, FRCPATH

Department of Clinical Pathology, Institute of Endemic Diseases, University of Khartoum

It was 45 years ago that Lynch and EL Hassan described a lymphoma in Sudanese children under the title, Multicentric sarcoma of the jaw', a tumor that was later to be known as Burkitt's lymphoma. Since then several malignant tumours in man that are suspected to be caused by EBV have been described in Sudan. These include nasopharyngeal cancer, Hodgkin's disease, Burkitt's lymphoma, and Angiocentric NK/T cell lymphoma. This presentation is about the latter two tumours in Sudan since our first publication on the subject. The majority of the cases of Burkitt's lymphoma in the sixties and seventies were of the endemic type with the majority involving the jaws. The cases were mainly in the holo- and mesoendemic malaria regions. In the last two decades there was a change in the pattern of the tumour. For example in a single centre between 2000 and 2005 of 33 Burkitt's lymphoma cases, only 4 involved the jaws. The epidemiology, clinical manifestations, pathology and possible causes for the change in the epidemiology will be described. Recently the virus has been demonstrated in the tumour by molecular biological methods. Four patients with Angiocentric NK/T cell lymphoma, another EBV related tumour, will be discussed. Three were nasal type; the fourth had a tumour involving the perianal region and an associated acute myeloid leukaemia.

HIGH GRADE B-CELL LYMPHOMAS OTHER THAN BURKITT LYMPHOMA

PATHOLOGY AND BIOLOGY

HIGH-GRADE B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA. WHO DEFINITION.

Nancy Lee Harris

Massachusetts General Hospital, USA

B-cell lymphomas with features intermediate between Diffuse Large B-cell Lymphoma (DLBCL) and Burkitt Lymphoma (BL) are defined in the WHO Classification (4th ed) as aggressive lymphomas with morphological and genetic features of both DLBCL and Burkitt lymphoma. This is not a reproducible category, but rather reflects the difficulty in categorizing some cases that are at the borderline between BL and DLBCL. Rather than force such cases into one category or the other, it is felt to be preferable to consider them unclassifiable until further information about these cases is available. The diagnosis should only be made using a combination of morphology with immunophenotypic and cytogenetic/molecular studies.
These lymphomas are composed of medium to large sized blastic cells that may mimic atypical Burkitt lymphoma, be intermediate in nuclear size between BL and DLBCL, resembling small centroblasts, or even resemble lymphoblastic lymphoma. Starry sky macrophages are often present, and mitotic figures and apoptosis are prominent. They express pan-B-cell markers; most are CD10 and bcl6 positive, and MUM1/IRF4 negative, similar to BL. Bcl2 may be positive. The Ki67 proliferation fraction is high (>90%).
About half of the cases have 8q24/MYC translocations. However, whereas in Burkitt lymphoma MYC is juxtaposed to the one of the immunoglobulin genes (MYC-Ig), many of these cases have other translocations (MYC-non Ig), and may have both BCL2 and MYC translocation (double hit). BCL6 translocations may be seen, sometimes with MYC and/or BCL2. Cytogenetic analysis often shows a complex karyotype, in contrast to classical Burkitt lymphoma. Gene expression profiling studies have found cases that are intermediate between BL and DLBCL, suggesting that there may be a true grey zone between these diseases. These are aggressive lymphomas, and the most appropriate therapeutic approach is not established.

IMMUNOHISTOCHEMISTRY EXPRESSION PROFILES AND SURVIVAL OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA IN UGANDA.

Lynnette K. Tumwine[1], Cristina Campidelli[2], Simona Righi[2], Sophia Neda[2], Wilson Byarugaba[1], Jackson Orem[3], Stefano A. Pileri[2]

[1]Department of Pathology, Makerere University Medical School, P.O.Box 7072, Kampala, Uganda. [2]Unit of Haematopathology, Institute of Hematology and Clinical Oncology "L & A Seragnoli, Bologna University School of Medicine, 40138 Bologna, Italy" [3]Uganda Cancer Institute, Kampala, Uganda.

Background: Diffuse large B cell lymphoma (DLBCL) is the second most common lymphoma in Uganda after Burkitt's lymphoma. It is heterogeneous in morphology and immunophenotype and recent studies in the developed countries have shown differences in survival. Since the onset of the AIDS pandemic it has been noted that more aggressive subtypes predominate here with rapid disease progression and very poor outcome.
Objective: To describe the different subtypes of diffuse large B cell lymphomas and correlate them to patient survival in Uganda.
Materials and methods: This study has two designs. A cross sectional descriptive design was used for subtyping and a retrospective cohort to determine duration of surivival. For the cross sectional study, Haematoxylin and eosin staining was carried out partly in the Department of Pathology, Makerere University and in the Unit of Hematopathology, Institute of Hematology and Clinical Oncology "L. & A. Seràgnoli", Bologna University School of Medicine, Bologna, Italy. Nineteen biopsies of patients diagnosed between 1991-2000 as non Hodgkin lymphoma were DLBCL after immunohistochemistry using CD3, CD5, CD10, CD20, CD23, CD30, CD38, CD79a, CD138, Bcl-6, Bcl-2, IRTA-1, MUM1/IRF4, Bcl-1/cyclin D1, TdT, ALKc, and Ki-67/Mib1. These patients were treated at the Uganda Cancer Institute with and their files were retrieved to obtained details of their home addresses and next of kin. A research assistant did retrospective follow up in their villages to establish how long they lived.
Results: DLBCL were subtyped into 9 Activated B Cell (ABC) type (CD20+, CD10-, Bcl-6-, Bcl-2+, MUM1/IRF4±, CD30+ or CD138+), 5 Germinal Centre B-cell (GCB) type (CD20+, CD10+, Bcl-6+, MUM1/IRF4±, Bcl2-), and 5 Unclassified (UC) type (CD20+, CD10-, Bcl-6+, Bcl-2+). The patients survival is summarised in the Kaplan and Meier survival curves.
Conclusion: Most 9 (47.4%) were Activated B Cell (ABC) type that has a bad prognosis.

PATTERNS OF LYMPHOMAS IN KENYA

Bessie Byakika, Lucy Muchiri, Wairimu Waweru, Kungu A.

Department of Pathology, The Nairobi Hospital, Nairobi, Kenya.

Objective: The objective was to report and compare the patterns of Lymphoma in a developing country with those in the developed world and also to form a data baseline for lymphomas in Kenya.
Study Design: A study was conducted to establish the pattern of lymphomas in Kenya. 125 lymphoma cases from both urban and rural Kenya were recruited into the study group. All cases were classified using the International Lymphoma Study Group guidelines. Immunophenotyping was performed for each tumour.
Results: The most frequent diagnosis was that of diffuse large B cell lymphoma (29.6%). The relationship with HIV infection was however not established. Hodgkin's lymphoma formed 25.6% with the nodular sclerosis being its most common variant. Burkitts lymphoma contributed 16% and was the most common Lymphoma seen in the pediatric age group with the average patient age of 6 years. Other Lymphomas formed small groups with Marginal Zone Lymphoma and Plasmablastic Lymphomas forming the larger group.
Conclusion: From the study the pattern of Lymphoma seen follow closely those documented in Western Literature. It would be however interesting to determine the genetic phenotypes of our disease.

IMMUNE ESCAPE AND PROLIFERATION: COMMON FEATURES OF BURKITT LYMPHOMA (BL) AND THE MOST AGGRESSIVE DIFFUSE LARGE B CELL LYMPHOMAS (DLBCL).

Lisa M. Rimsza MD[1], Joseph M. Unger MS[2], Randy D. Gascoyne MD[3], Robin A. Roberts BS[1], Daniel O. Persky MD[4], Thomas P. Miller MD[4], Joseph M. Connors MD[3], Laurie H. Sehn MD[3], Michael L. LeBlanc PhD[3]

[1]Department of Pathology, University of Arizona, Tucson, Arizona, USA
[2]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA
[3]British Columbia Cancer Agency, Vancouver, British Columbia, Canada
[4]Arizona Cancer Center, Tucson, Arizona, USA

Gene expression profiling studies of BL and DLBCL revealed key pathogenic and prognostic signatures. In BL, C-MYC based Proliferation and loss of Major Histocompatibility (MHC) Class I define adverse features. In DLBCL treated with CHOP chemotherapy, key signatures are Proliferation (including C-MYC, adverse), MHC Class II, Germinal center B cell, and Lymph Node. We questioned whether these 4 DLBCL signatures and other key genes were relevant in the R-CHOP treatment era and if a new gene-risk model of DLBCL could be designed.
Using a quantitative nuclease protection assay that measures expression levels in formalin fixed, paraffin embedded tissues (ArrayPlate®, High Throughput Genomics, Tucson, AZ), we quantified 36 prognostic genes from 101 DLBCL patients treated with R-CHOP. Cox Proportional Hazard Models defined the best 1, 2, 3, and 4 variable gene models to predict patient outcome.
9 of 36 genes were significantly associated ($p<0.05$) with survival including SERPINA9, HLA-DQA, HLA-DRB, PLAU, C-MYC, BCL6, LMO2, PDCD4, and SOD2. These genes were represent the 4 DLBCL signatures: Proliferation (C-MYC), MHC Class II (HLA-DQA, HLA-DPB), Germinal Center (SERPINA9/GCET2, BCL6), and Lymph Node (PLAU). The best 2-variable model was the combination of C-MYC and HLA-DRB (chi-square 19.6). When patients were defined as having high C-MYC or low HLA-DRB, 23 patients (23%) had both adverse factors. These patients had worse survival than those with 0-1 factors (2-year OS 42% vs. 84%). Adjusting for IPI, this difference was highly significant ($p=.004$).
In the R-CHOP era, the 4 signatures retain prognostic value. Loss of MHC expression with increased C-MYC defined the patients with the worst outcome. It is striking that these are also the defining attributes of BL suggesting that lack of immunosurveillance and high proliferation are the key features defining the most aggressive B cell lymphomas.

B-CELL LYMPHOMA WITH CONCURRENT *C-MYC* AND *BCL2* TRANSLOCATIONS HAS CLINICOPATHOLOGICAL FEATURES DISTINCT FROM ATYPICAL URKITT LYMPHOMA

Matija Snuderl[1], Olga K. Kolman[1], Judith A. Ferry[1], Ephraim P. Hochberg[2], Yibin Chen[2], Robert P. Hasserjian[1] and Aliyah Rahemtullah[1]

Departments of [1]Pathology and [2]Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States.

Introduction: B-cell lymphoma (BCL) with concurrent CMYC and BCL2 translocations generally has a poor prognosis. We sought to characterize this neoplasm further to help distinguish it from atypical Burkitt lymphoma (ABL).
Design: 12 cases of BCL with concurrent t(8q24) and t(14;18) identified by conventional karyotype and/or FISH were diagnosed in our practice from 2004-2007. Clinicopathological data were compared to 9 ABL cases.
Results: The 6 men and 6 women had a median age of 62 yrs (vs 41 yrs in ABL group, $p<.001$). None were HIV+. 2 patients had a history of low-grade follicle center lymphoma (FCL). 7 cases resembled ABL, 4 resembled diffuse large B-cell lymphoma (DLBCL), while 1 case with a history of low-grade FCL showed diffuse FCL with blastoid features. Secondary involvement of bone marrow and CNS was pathologically confirmed in 5/10 and 4/8 cases respectively. All cases expressed ≥1 B-cell marker and were CD10+ and/or Bcl6+. 10/11 cases were Bcl2+ (vs 0/9 ABL cases, $p<.001$). 7/12 cases were Mum1+ (vs 1/9 ABL cases, $p=.06$). Ki-67 proliferation index (PI) ranged from 65-100% and was >95% in 3/12 cases (vs 9/9 ABL cases, $p<.001$). No case was EBER+. Full karyotype available in 6 cases was complex with ≥3 aberrations. 11 patients received combination anthracycline-based chemotherapy. Of 4 who achieved a complete response (CR), 3 relapsed within 6 m. After a median follow-up of 4 m, 9 patients died of disease, 2 were alive with disease undergoing chemotherapy and 1 was in CR.

Conclusion: Our study confirms poor outcomes despite intensive chemotherapy in BCL with concurrent CMYC and BCL2 and translocations. Distinguishing clinicopathologic features from ABL of statistical significance include older age at diagnosis, PI < 95% and Bcl2 positivity, with a trend toward significance seen for Mum1 positivity. Other potentially helpful features in diagnosing this rare neoplasm include absence of HIV and/or EBV as cofactors, DLBCL morphology and a complex karyotype.

MISMATCH REPAIR DEFICIENT LYMPHOID PROLIFERATIONS OCCUR THROUGH A FIELD CANCERIZATION DEFINED BY O-6-METHYLGUANINE-DNA METHYLTRANSFERASE LOSS OF EXPRESSION.

Alex Duval, Claire Borie, Martine Raphael

INSERM-UPMC, France

O6-methylguanine-DNA methyltransferase (MGMT) repairs DNA adducts that are mutagenic, favouring transitional events, and toxic by leading cells to apoptosis through a mismatch repair (MMR) dependent pathway. Cells whose MMR system is inactivated can thus tolerate O6-methylguanine in their DNA. The role of MGMT in human carcinogenesis has been poorly investigated. Here we report the frequent loss of expression of this enzyme due to methylation of its promoter site in a subset of lymphomas, i.e. post-transplant lymphoproliferative disorders (PTLD). Patients whose lymphomas had MGMT deficiency also exhibited frequent loss of MGMT expression in tumour-infiltrating normal lymphoid cells, defining a field defect by MGMT deficiency in these tumours. Of interest, a highly significant association between MGMT deficiency and inactivation of the MMR system was observed in PTLD as well as in a series of lymphoid cell lines (LCL). MMR deficiency notably occurred through deleterious transition mutations within MMR genes in these tumours. Accordingly, MGMT loss of function is highlighted as an early event favouring the transformation of lymphoid cells through MMR deficiency. These results have important implications for the management of grafted patients who are at risk of developing MMR deficient lymphomas.

BRD2 GENE AND PROTEIN EXPRESSION IN MALIGNANT LYMPHOMA.

Faisal Fadlelmola, Minglong Zhou, Ronald J. de Leeuw, Pedro Farinha, Christian Steidl, Randy Gascoyne, Wan Lam, and Diponkar Banerjee

Centre for Translational and Applied Genomics, Department of Pathology, British Columbia Cancer Agency, Vancouver, Canada and BC Cancer Research Centre.

BRD2 (bromodomain-containing protein 2), formerly known as RING3 (really interesting new gene 3) encodes an 85 kDa mitogen-activated kinase, Brd2, a transcription regulator, which localizes to the nucleus and acts as a scaffold for various transcription factors. Transgenic mice with lymphoid tissue-restricted overexpression of Brd2 develop a splenic B cell lymphoma which resembles human diffuse large B cell lymphoma in gene expression profile. Through gene expression profiling we showed increased expression of BRD2 in Hodgkin Lymphoma (HL) cell lines KMH2, L428 and L-1236 cells, confirmed by conventional RT-PCR and quantitative RT-PCR. Amplification of BRD2 was detected by submegabase resolution tiling array CGH (SMRT Array CGH) in L-1236 but not KMH2 or L428 cells. In 3 of 5 clinical samples of HL, microdissected Reed-Sternberg cells showed gains in the BRD2 locus. Immunohistochemistry for Brd2 was carried out on tissue microarray sections of Classical HL in comparison to diffuse large B cell lymphoma (DLBCL). In HL (n=50), 90% of cases showed nuclear positivity in Reed-Sternberg cells, while in DLBCL (n=83), 94% of cases showed nuclear positivity in large neoplastic B cells. The intensity of staining varies from 1+ (~90% of positive cases) to 3+ (~10% of positive cases). In reactive lymph nodes only cytoplasmic Brd2 protein expression was found in germinal centre centrocytes and centroblasts, apoptotic germinal centre cells, mantle zone cells, and in plasma cells. No nuclear staining was seen. Since nuclear Brd2 localisation is indicative of Brd2 activation, these observations indicated that germinal centre centrocytes, mantle zone cells and plasma cells lack Brd2 activation but neoplastic B cells in HL and DLBCL contain activated Brd2. Pathways associated with Brd2 activation will be explored in future studies. Funded by the Canadian Institutes of Health Research (CIHR) and the Lymphoma Foundation Canada.

PREVALENCE OF MYC REARRANGEMENTS IN GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA: INTERPHASE FISH ANALYSIS USING PARAFFIN-EMBEDDED BIOPSY SAMPLES.

Hidekazu Kayano[1], Toshinori Nagai[1], Mark E Law[2], Ellen D Remstein[2], Takanori Hirose[1]

[1]Department of Pathology, Saitama Medical University, Moroyama, Saitama, Japan. [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Background: Diffuse large B-cell lymphoma (DLBCL) has a heterogeneous molecular basis; MYC gene abnormality is seen in approximately 15% of DLBCL, but BCL2 or BCL6 genes are more frequently rearranged. In contrast, Burkitt lymphoma (BL) is characterized by constitutive rearrangement of MYC and immunoglobulin (IG) genes with paucity of BCL2 or BCL6 rearrangements. MYC rearrangements with several non-IG genes in DLBCL have recently been reported; in particular, MYC rearrangement without IG co-migration is frequently detected in gastric DLBCL. Practically, histological distinction of DLBCL from BL occasionally has to be done in gastric biopsies, but molecular analysis is limited due to difficulties in obtaining sufficiently large, fresh biopsy samples. Methods: We conducted interphase FISH analysis on sections of paraffin-embedded gastric biopsy specimens confirmed as DLBCL. Subjects comprised 10 adult patients (age, 55 to 87 years). The following FISH probes were used: 2 break apart rearrangement probes for MYC and BCL6 genes, and 4 dual fusion translocation probes for MYC/IG fusion (heavy chain (MYC/IGH) and light chains (MYC/IGK and MYC/IGL)) and IGH/BCL2 10% of nuclei from 200-250 tumor cells³fusion. Abnormal signals in were considered to indicate a positive result. Results: Rearrangement of MYC, BCL6 and BCL2 was detected in 4, 3 and 2 cases, respectively. MYC rearrangements were associated with IGH/BCL2 fusion (two cases) and/or BCL6 rearrangement (two cases). A higher percentage of cells had BCL2 and/or BCL6 rearrangements than MYC rearrangement, suggesting that MYC aberrations are a secondary event.
MYC/IGL translocation was found in another case. No MYC/IGH or IGK fusions were observed in this series. Conclusions: FISH analysis may be useful in distinguishing DLBCL from BL on small gastric biopsy samples. Our results suggest that MYC rearrangements in gastric DLBCL may be secondary genetic events and usually involve non-IG partner genes.

DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WITH T(14;18) AND 8Q24/C-MYC REARRANGEMENT IS A LYMPHOID NEOPLASM WITH AGGRESSIVE CLINICAL PRESENTATION AND VERY POOR PROGNOSIS.

Steven Le Gouill*[1], Pascaline Talmant*[2], Cyrille Touzeau[1], Anne Moreau[3], Richard Garand[2], Nadine Juge-Morineau[4], Fanny Gaillard[3], Thomas Gastinne[1], Noël Milpied[5], Philippe Moreau[1], Jean Luc Harousseau[1] and Hervé Avet-Loiseau[2]

[1]Department of Hematology, Hôtel Dieu, University Hospital of Nantes, Place Alexis Ricordeau, 4093 Nantes cedex 01, France; [2]Laboratory of Hematology, Clinical Hematology Department, University Hospital, Place Alexis Ricordeau, 44093 Nantes cedex 01, France; [3]Laboratory of Anatomopathology, University Hospital, Place Alexis Ricordeau 44093 Nantes cedex 01, France; [4]Centre Catherine de Sienne, 2 rue Eric Tabarly, 44200 Nantes, France; [5]Department of Hematology, University Hospital, Hôpital Haut Lévèque, 3604 Pessac, France

We identified 16 Diffuse Large B Cell Lymphomas (DLBCLs) with concurrent t(14;18) and 8q24/c-MYC rearrangement and describe their clinical, biological, immunophenotyping and cytogenetic features. All patients had aggressive features: B symptoms (81%), ECOG PS > 2 (81%), elevated LDH (100%), stage IV disease (100 %) with at least one extra nodal localisation (bone marrow, blood and CNS involvement in 93%, 50% and 50%, respectively) and age-adjusted IPI = 3 in 81%. Despite intensive chemotherapy regimens (including allogeneic transplants), all patients died because of disease progression. PFS and OS were 4 and 5 months, respectively. Immunophenotyping analysis (CD20, CD10, Bcl-6, Mum-1, Bcl-2 CD138, MIB1, CD19, CD5, CD38 and sIg) was performed and showed DLBCLs with a germinal center (GC) profile. Ki-67 staining ranged from 70 to 90%. All cases assessed by cytogenetic analysis (conventional cytogenetic (CC) and/or FISH analysis) had a complex karyotype. In one case, we identified a 8q24/c-MYC translocation variant never reported in DLBCLs before : t(8;9)(q24;p13) and t(14;18)(q32;q21). BCL-6 rearrangement was investigated (FISH) and found rearranged in 4 cases. In conclusion, DLBCLs with

concurrent t(14;18) and 8q24/c-MYC rearrangement is subgroup of GC-DLBCLs. Coexistence of dual translocation is worth being searched in Bcl-2 positive DLBCLs with unusual aggressive presentation.

TRANSFORMATION OF FOLLICULAR LYMPHOMA TO BURKITT-LIKE LYMPHOMA WITHIN A SINGLE LYMPH NODE

Jagmohan S. Sidhu MD[1], Sanjay Mukhopadhyay MD[2], Joseph Readling MD[3], Philip Cotter PhD FACMG[4], Anthony E Shrimpton PhD[5]

[1]Department of Pathology, United Health Services Hospitals, Johnson City, NY, 13790. [2]Department of Pathology, SUNY Upstate Medical University, Syracuse, NY, 13210. [3]Department of Oncology, United Health Services Hospitals, Johnson City, NY, 13790. [4]Cytogenetics and Molecular Genetics, US Laboratories, Irvine, CA, 92612. [5]Department of Pathology, SUNY Upstate Medical University, Syracuse, NY, 13210.

Aberrant expression of *bcl-2*, caused by a t(14;18) translocation, most commonly occurs in follicular lymphoma (FL). In some of these tumors, additional acquisition of a translocation involving *c-myc* leads to transformation to a high-grade lymphoma, usually to diffuse large B-cell lymphoma. Transformation of FL to Burkitt-like lymphoma (BLL) is uncommon. We encountered a case of FL containing a t(14;18) translocation transforming into a BLL containing the original t(14;18) as well as an additional t(8;14). The latter translocation resulted in the phenotype of BLL and transformation from FL to BLL was demonstrable within a single lymph node in the form of three distinctive components in continuity with each other. The first component consisted of follicles, some showing a predominance of centrocytes and others showing an admixture of centrocytes and centroblasts. The second component consisted of medium-sized lymphoid cells (Butkitt cells) arranged in a follicular pattern, with a "starry-sky" appearance, brisk mitosis, and abundant apoptosis. The third component consisted of cells similar to the second component but arranged diffusely. Both the FL and the BLL were positive for CD20, CD79a, *bcl-2*, *bcl-6*, and showed κ light chain restriction. The Ki-67 labeling index was low (25%-50%) in the FL and almost 100% in the BLL in both the follicular and diffuse areas. Fluorescent in situ hybridization (FISH) of the FL region was positive for t(14;18) and negative for t(8;14). FISH of the Burkitt-like region with follicular pattern was positive for both t(14;18) and t(8;14). Similarly, the Burkitt-like region with diffuse pattern was positive for both t(14;18) and t(8;14). To the best of our knowledge, this is the first description of a case documenting direct transformation of FL into BLL in the same lymph node. This case illustrates the dramatic oncogenic stimulus that results from the inhibition of apoptosis by *bcl-2* combined with the deregulation of cell growth by *c-myc*.

FREQUENCY AND FEATURES OF THE CMYC REARRANGEMENTS (DETECTED BY FISH AND ARRAYCGH) IN OTHER B CELL NEOPLASIAS: THE EXAMPLE OF MULTIPLE MYELOMA

Bibiana I. Ferreira, Cristina Largo, Juan C. Cigudosa

Molecular Cytogenetics Group, Centro Nacional de Investigaciones Oncologicas (CNIO). Madrid. Spain.

Text: Chromosome translocations involving CMYC oncogene and the loci of Immunoglobulin gene constitute the genetic hallmark of Burkitt lymphomas. However, such translocation and other rearrangements of CMYC have also been described in B cell tumours that do not belong to this specific category. We and others have described that CMYC translocation and genomic gains are frequent in multiple myeloma (MM) (1,2). It is assumed that CMYC plays a significant role as a secondary genetic aberration in MM.
Objective: To study of the frequency and the nature of CMYC rearrangement in MM.
Material and Methods: (1) A home made TMA containing 31 primary samples of MM was subjected to FISH analysis. We used the CMYC breakapart probe (BA) that has been developed by DAKO (Cat # Y5410). FISH assay was carried with standard protocols as provided by the supplier. (2) An additional panel of 27 MM cases were analyzed for genomic profiling (by arrayCGH). ArrayCGH was performed in a Human Genome CGH Microarray 44B platform from Agilent Technologies (Palo Alto, CA). This platform contains 44000 60-mer oligonucleotides covering the genome with an average resolution of 45 Kb. CGHAnalytics and InSilicoArray were used for array analysis.
Results: 23 out of 31 cases were successfully analyzed by FISH. Six cases (19%) showed CMYC rearrangements. Four cases could be diagnosed as positives for the translocation (separated signals with the BA CMYC probe) and two more cases showed three copies of the gene. In the arrayCGH study, three cases (11%) showed gain of the CMYC genomic region. The discrepancy of the percentage is due to the limitation of the arrayCGH technique detecting translocations.
Refs:

1. Largo et al. Identification of amplified/overexpressed genes in multiple myeloma. Haematologica. 2006, 91:184
2. Keatts et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. Cancer Cell. 2007,12:131

CHLAMYDIA INFECTION AND THE DEVELOPMENT OF THE OCULAR ADNEXAL LYMPHOMAS

A. Carugi[1], A. Onnis[1], M. Cocco[1], A. Luzzi[1], J. Nyagol[4], G. Cerino[1], S. Shaheen[4], GM. Tosi[2], C. Bellan[1], G. De Falco[1,3], A. Giordano[1,3], L. Leoncini[1]

[1]Dpt. Human Pathology and Oncology, University of Siena, Italy; [2]Dpt. of Ophtalmology, University of Siena, Italy; [3]Sbarro institute for Cancer Research & Molecular Medicine, Temple University, Philadelphia PA 19122, USA; [4]Dpt of Pathology, Aga Khan University Hospital, Nairobi-Kenya

Non-Hodgkins lymphomas develop from nodal and extra-nodal tissue. A particular extra-nodal lymphoma type arises from B cells of the marginal zone (MZ) of mucosa-associated lymphoid tissue (MALT). The geographic heterogeneity in the incidence of B-cell non Hodgkin's lymphomas and the growing evidence suggest that MZ lymphomas are associated with chronic antigenic stimulation by microbial pathogens, among which *H. pylori*-associated gastric MALT lymphoma is the best studied.
Recently, MALT lymphomas have been described in the context of chronic conjunctivitis, which can be associated with *Chlamydia* infection. Studies from Italy showed *Chlamydia psittaci* in 87% of ocular adnexal MALT lymphomas and complete or partial regression of the lymphoma after *C. psittaci* eradication in four of nine cases. However *C. psittaci* was not founded in ocular adnexal lymphoma from other studies. As association with *C. psittaci* does not seem to be a constant parameter, this variability may depend on geographic heterogeneity.
This project was designed to further investigate the role of *Chlamydia psittaci* in the development of ocular adnexal MALT lymphomas, by comparison of cases retrieved from different geographic areas, as Kenia and Italy.
The presence of *C. psittaci* DNA in biopsies of ocular adnexal lymphomas was demonstrated by TETR-PCR, a modified PCR reaction, used to amplify different DNA sequences in the variable regions of the 16S and 23S spacer rRNA genes specific for *Chlamydia psittaci*.
DNA was extracted from 31 ocular adnexal lymphomas, retrieved from two different geographical regions. The prevalence of *C. psittaci* infection in MALT lymphoma showed a marked variation between the two geographical regions. 20% (5/25) of the samples from Italy were positive for *C. psittaci*, but no association with this pathogen was observed in any of the samples from Kenya.
Furthermore, we investigated a possible relationship between *C. psittaci* infection and the promoter hypermethylation of p16/INK4a. This epigenetic alteration has been described in *H. pylori*-associated gastric MALT lymphoma. Our results show a partial methylation of p16/INK4a promoter in 46% (12/26) of *C. psittaci*-negative cases, whereas no hypermethylation of this gene was found in *C. psittaci*-positive cases. As genetic alterations, as the t(11;18) and t(1;14), have been described in MALT lymphomas, we are currently performing FISH studies to evaluate whether such genetic alterations may also occur in adnexal lymphomas, besides *Chlamydia* association.
From these findings, we may conclude that many and different infectious agents may play a critical role in MALT lymphoma development.

SERO-PREVALENCE OF HEPATITIS VIRUSES IN NON-HODGKIN'S LYMPHOMA IN OMAN

Ikram A Burney, and Mansour S Al Moundhri.

Medical Oncology unit, Department of Medicine, Sultan Qaboos University Hospital, Muscat 123, Oman.

There is a growing body of evidence suggesting the possible role of hepatitis C virus (HCV) in the etiology of Non-Hodgkin's Lymphoma (NHL). A high rate of association between HCV and NHL has been reported especially from areas of high endemicity. The data on Hepatitis B virus (HBV) is less

definitive. Oman is one of the, and there are no data on the association of viruses in NHL. This study addresses the issue of sero-prevalence from Oman. The data were collected retrospectively from consecutive adult patients (>14 years) diagnosed to have NHL between June 1999 and Dec 2006, and the sero-prevalence of HBV and HCV was compared with the healthy blood donors. The diagnosis was established according to the WHO classification. Patients with ALL and CLL were excluded. Data were available from a total of 94 patients. Median age was 49 (14-86) years. There were 61 males and 33 females. 59% patients had DLBCL, 8.5% had ALCL, and 8.5% had FL. 6/94 (6.5%) showed positivity for Anti-HCV, 9/94 (9.4%) were positive for HepBsAg, whereas, 26/54 (48%) were positive for total core antibody. Four patients had concomitant HIV infection. The sero-prevalence rate for HCV and HBV in the control group were 1.2% and 4.9% respectively. There were no significant differences in either the clinico-pathological features or the overall survival among patients who were either HBV/HCV positive or negative. Sero-prevalence rates for HBV from different reported studies are shown as under:

Country	Reported	Cases/positive	Controls/positive	Significance
Romania	1999	68 (30.8%)	943 (6.3%)	p<0.001
Japan	2001	348 (6.9%)	1,513,358 (?)	p=0.05
Japan	2005	218 (7.3%)	(1.2%)	p<0.05
Italy	2006	400 (8.5%)	392 (2.8%)	p<0.05
Turkey	2006	203 (14.5%)	NR(8.2%)	
Singapore	2006	556 (10.3%)	4698 (4.1%)	p<0.001
Current Study	2008	94 (9.4%)	4.9%	p<0.05

In conclusion, the sero-prevalence rate for both HBV and HCV is higher in patients with NHL compared to the controls in Oman.

THE CURRENT STATE OF LYMPHOMA HISTOPATHOLOGY IN UGANDA

S. Kalungi[1,2], A. Molven[2], H.R. Wabinga[1], L. Bostad[2]

[1]Department of Pathology, Faculty of Medicine, Makerere University, Uganda [2]The Gade Institute, Section for Pathology, University of Bergen, Haukeland University Hospital, Bergen, Norway

Introduction: The epidemiology of haematopoietic and lymphoid tumours continues to pose a challenge in the developing countries. The lack of adequate clinical and demographic information is part of the problem. During the last twenty years there have been a number of different classification systems also causing problems in this setting. Modern lymphoma classification requires ancillary techniques that may be unavailable.
Objective: To look at the standard of hematopathology reporting and examine the frequency and diagnostic profile of lymphomas diagnosed in Uganda in the period 1980-1989
Setting: Department of Pathology, Faculty of Medicine, Makerere University, Kampala, Uganda.
Methods: Review of the patients` pathology records for the years 1980-1989 from the files of the Department of Pathology, Makerere University.
Results: A total of 1013 patients were diagnosed with lymphoma in the period 1980-1989. The most common type of non-Hodgkin lymphoma was Burkitt lymphoma (36%). The frequencies of lymphocytic, histiocytic and other histological types were 34%, 8% and 22%, respectively. Light microscopy of Haematoxylin and Eosin stained slides is the only method used for lymphoma diagnostics in Uganda.
Conclusion: Burkitt lymphoma continues to be the most common subtype of lymphoma diagnosed in Uganda. Some lymphoma subtypes are not reported in the country in this period. Modern lymphoma classification is based on a multimethodological approach and includes the clinical picture, morphology, immunophenotype and molecular genetic features. Accordingly,quality improvement in this field of surgical pathology requires implementation of ancillary laboratory techniques

TRANSFORMED DIFFUSE LARGE B-CELL LYMPHOMA OF GERMINAL CENTER ORIGIN APPEARING „BURKITT-LIKE“ ON MORPHOLOGIC AND TYPE 3 (NON-GC/NON-ABC TYPE)ON IMMUNOHISTOCHEMICAL EXAMINATION

Jagmohan S. Sidhu MD[1], Madhuri Yalamanchili MD[2] and Peter R. Papenhausen PhD[3]

[1] and [2]UHS Hospitals, Department of Pathology and Laboratory Medicine, Johnson City, NY, USA (affiliated with SUNY Upstate Medical University, Syracuse, NY). [3]Laboratory Corporation of America,Center for Molecular Biology and Pathology, Research Triangle Park, NC,USA.

We performed immunohistochemical and interphase cytogenetic analysis using fluorescence in situ hybridization (FISH) on a case of transformed diffuse large B-cell lymphoma (DLBCL) with „Burkitt-like‘ morphology in order to confirm transformation and to rule-out/rule-in Burkitt lymphoma (BL). These studies were performed on a cervical lymph node excision specimen of a 64-year-old male who had developed cervical lymphadenopathy a few years after a diagnosis of low-grade follicular lymphoma was made on an inguinal lymph node biopsy. He was not treated for his low-grade lymphoma. The cervical lymph node biopsy showed 100% diffuse infiltrate of medium to large sized lymphoid cells with 1-2 prominent nucleoli, numerous mitoses, marked apoptosis, and „starry-sky“ appearance created by „tingible-body“ macrophages. About 90% of the lymphoid cells were positive for CD20, CD10, BCL2, BCL6, and Ki67. MUM1 was negative. This immunophenotype is neither of germinal center (GC) type nor of activated B-cell (ABC) type and is, therefore, of type 3 DLBCL. Flow cytometric analysis of the lymph node showed monotypic B-cell population with surface expression of CD19, CD20, CD23 (dim), CD38, and lambda light chain. FISH for c-MYC gene rearrangement and BCL2 gene rearrangement was performed on the formalin fixed paraffin-embedded tissue, which showed c-MYC gene rearrangement in about 44% of nuclei in an apparent tetraploid clone, BCL2 gene rearrangement in about 86% of nuclei, and an extra BCL2 gene signal additional chromosome 18) in most of the nuclei with BCL2 gene rearrangement. Additional chromosome 18 supports transformed DLBCL and the presence of c-MYC gene rearrangement only in about 44% nuclei rules-out the possibility of BL. Our case emphasizes the value of looking at the percentage of c-MYC gene arrangement positive nuclei as a way of differentiating „Burkitt-like“ DLBCL from BL and also shows that some cases of apparent type 3 DLBCL can actually be transformed GC type DLBCL.

CLINICAL ASPECTS, CHEMOTHERAPY AND NOVEL THERAPEUTIC TARGETS

IS INTENSIVE CHEMOTHERPAY FOR AGGRESSIVE AND HIGHLY AGGRESSIVE NON-HODGKIN'S LYMPHOMA NECESSARY IN AFRICA, AND IS IT FEASIBLE?

NA Othieno Abinya – MB, ChB M'Med

Department of Clinical Medicine and Therapeutics, School of Medicine, College of Health Sciences, University of Nairobi.

Aggressive and highly aggressive phenotype non-Hodgkin's lymphomas (NHLs) were categorised as intermediate and high grade variants in the International Working Formulation. Whereas this categorization is not easily applicable wholesome in the current widely used World Health Organization (WHO) update of the Revised European/American Lymphoma (REAL) classification for lymphoid neoplasms, lymphoma types considered are diffuse large B-cell lymphoma, variants of mantle cell lymphoma, variants of lymphoplasmacytic lymphoma, angioimmunoblastic T-cell lymphoma, enteropathy-type T-cell lymphoma, peripheral T-cell lymphoma, unspecified, adult T-cell leukaemia/lymphoma (ATL). Others in the highly aggressive category include sub variants of diffuse large-B cell lymphoma, precursor B- and T-cell lymphoblastic leukaemia/lymphoma, Burkitt's lymphoma, sub variants of peripheral T-cell lymphoma-unspecified, angioimmunoblastic T-cell lymphoma, enteropathy-type T-cell lymphoma, sub variants of anaplastic large-cell lymphoma, primary-systemic type, sub variants of ATL.
Treatment of these lymphomas has evolved over the years from the predominant use of single agent chemotherapy with 5 year survivals being virtually zero. When the combination of C-MOPP protocol was introduced in the 1960s by workers from the US National Cancer Institute, complete remission (CR) rates of 45% were realised, with most patients who achieved CR remaining disease-free 24 years later. The cure rates were estimated at 37%, a far cry from the CR rates of 73-80% with cure rates of over 60% achiev-

able with the current intensive chemotherapies that incorporate high dose salvage protocols, with or without monoclonal antibody therapy in patients with advanced disease.
Africa today is still at the stage where industrialised countries were in the 1960s and 1970s. The problem is not lack of technology, drugs or support requirements. These can be imported. The problem is the weak economies that make it difficult to purchase the required medicines and equipment. The gross national income per-capita for Kenya is 1396 US Dollars not different from most of Africa. Here 46% of the population survive on less than one US Dollar per day. The cost of medication required for the cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) protocol plus effective anti-emetics for example is 450 US Dollars and is not affordable for the majority of the population in countries like Kenya with total expenditure on health per capita of 8.3 US Dollars. From this perspective it is prudent to limit Africa to tailored protocols that are affordable. After all something is always better than nothing. The problem is, the protocols such as those used in the 1960s and 70s were basically palliative and left large chunks of the population with heavy disease burdens.
Africa has enough resources but proper resource utilization is wanting. Rather than give up and subject everyone including some of the most deserving cases to treatments that are basically palliative, it is better to apply the rule 'what is good for Europeans and Americans is also what is good for Africans', and encourage policy makers to practise good governance and proper utilization of resources. An intensive protocol dubbed BEMACOPPA is undergoing phase II trials in Nairobi, though accrual rate is slow.

OPTIMIZING CHEMOIMMUNOTHERAPY IN AGGRESSIVE LYMPHOMAS.

Lorenz Trümper

Georg August University of Göttingen, Germany

Aggressive lymphoma comprises a group of clinically and biologically heterogeneous diseases characterized by rapid growth of lymphoma cells and good response to chemotherapy with CHOP-based regimens, immunotherapy with CD20 antibodies and radiotherapy. Recently, gene expression and IHC based classifiers have been devised, based on retrospective patient groups. In the clinic, the easily applicable international prognostic index is prognostically valid. Data modifying this index have been presented by Sehn et al (Vancouver group); data of our group based on the Ricover-60 collective still uphold the validity of this index (Ziepert et al).
Standard treatment has been improved by dose density approaches utilized by our group (Pfreundschuh, Trümper et al., 2004) and by the addition of Rituximab (Coiffier et al, 2002; Pfreundschuh al, 2006). The Ricover-60 trial compared in a bifactorial fashion 6 vs 8 cycles of CHOP and R-CHOP vs CHOP alone in a dose dense fashion (Pfreundschuh et al., submitted).
R-CHOP-14 for 6 cycles is the standard regiment for elderly patients for the DSHNHL. Further trials by GELA and HOVON will be presented in the near future. Based on initial Rituximab-denser treatments by the SWOG, the DSHNHL has started phase II trials with denser applications of Rituximab at the initiation of treatment, thereby improving responses. This regiment will now be tested in a phase III trial. Further escalation of chemotherapy by dose-dense high dose chemotherapy has not been shown to be superior to standard CHOP-based therapy so far. The results and reasons will be discussed in detail. Allogeneic transplantation modified by antibodies has gained a prominent role in the relapse treatment of aggressive lymphomas. Results of the DSHNHL R3 trial will be updated. In summary, clinical approaches have greatly diversified over the last years, allowing treatment strategies based on individual risk factors. Improved classifiers based on biological differences are needed.

NOVEL TARGETED THERAPEUTIC APPROACHES FOR THE TREATMENT OF VIRAL LYMPHOMAS.

Ethel Cesarman

Department of Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, NY, USA

Epstein-Barr virus (EBV), Kaposi's sarcoma herpesvirus (KSHV), also called Human Herpesvirus 8 (HHV-8), and human T-cell lymphotrophic virus (HTLV-1) are viruses that are well documented to be causally associated with lymphoid neoplasia in humans. Other viruses have also been proposed to be involved in lymphomagenesis, but their role may be indirect, or the association is not well established. Current knowledge suggests that EBV, KSHV and HTLV-1 contribute to lymphomagenesis by subverting the host-cell molecular signaling machinery to deregulate cell growth and survival. For example, deregulation of the NF-kB pathway is a common strategy used by all three three lymphomagenic viruses to promote cell survival, thereby playing a critical role in tumorigenesis. Therefore, new drugs that inhibit NF-kB may be beneficial for the treatment of viral lymphomas, alone or in combination with chemotherapy. Other cellular pathways that may represent good therapeutic targets as they are deregulated in viral malignancies include cMYC, p53 and mTOR. Inhibitors for these molecules are in development or are currently in clinical trials for other malignancies. Recent data indicates that at least in some viral lymphomas, the elimination of a single viral protein inhibits the proliferation of tumor cells or leads to their apoptosis in vitro. This has been shown for EBV EBNA1, LMP1 and LMP2, and for KSHV vFLIP and vIRF-3 genes using RNA interference. This gives us the unique and exciting opportunity to target these viral proteins for the treatment of the malignancies associated with specific infections. Targeting of viral proteins involved in oncogenesis would be advantageous over inhibiting cellular proteins, as therapy could be completely specific and non-toxic.

CANNABINOID RECEPTORS AS MEDIATORS OF CELL DEATH IN NON-HODGKIN´S LYMPHOMA

Kristin Gustafsson[1], Xiao Wang[1], Maeve Eriksson[1], Denise Severa[1], Eva Kimby[2], Mats Merup[2], Birger Christensson[1], Jenny Flygare[1], Birgitta Sander[1]

[1]Department of Laboratory Medicine, Division of Pathology, F46 Karolinska Institutet and Karolinska University Hospital Huddinge, SE 141 86 Stockholm, Sweden.

In previous studies we identified the cannabinoid receptor type 1 (CB1) and type 2 (CB2) as over expressed in mantle cell lymphoma (MCL). Endogenous and synthetic cannabinoids inhibited proliferation and induced apoptotic cell death in MCL while normal B cells were spared; suggesting that targeting of cannabinoid receptors could provide a new treatment option in MCL. In the present study, expression of CB1 and CB2 was evaluated in a broad panel of indolent and aggressive B lymphocyte malignancies (n=62). A majority of the B cell lymphomas expressed higher levels of CB1 and CB2 than reactive lymphoid tissue. In contrast to MCL, that uniformly express CB1 and CB2, the expression in other malignant B cell lymphomas was highly variable. There was a significant correlation between the expression of CB1 and CB2 in malignant lymphomas. Low levels of the splice variant CB1a, previously shown to have a different affinity for cannabinoids than CB1, was detected in 30% of CB1 expressing lymphomas. In functional studies, using cell lines derived from MCL, Burkitt lymphoma, CLL and plasma cell malignancies, cannabinoids induced cell death in cells expressing both CB1 and CB2. Finally, in mice xenotransplanted with human MCL tumors, there was a significant reduction in mitotic index and tumor size in cannabinoid treated mice compared to control mice. Our studies demonstrate that cannabinoid receptors are widely expressed in malignant lymphoma. Thus, targeting of the endocannabinoid system might provide a new mode of treatment in malignant lymphoma.

RESULTS OF BURKITT'S LYMPHOMA AND OTHER AGGRESSIVE B-CELL MALIGNANCY BY REVISED PROTOCOL NHL-BFM-90: SINGLE CENTRE EXPERIENCE.

Semochkin S.V., Loriya S.S., Rumiantsev A.G.

Federal Scientific Clinical Center for Pediatric Hematology,Oncology and Immunology, Moscow, Russia

Patients and methods: 57 (m-40, f-17) de novo patients (pts) have been enrolled in this study during 1990-2005. Treatment under the revised protocol NHL-BFM-90 has been conducted to 34 (60%) pts (median age 12.7, range 10-61 years) with Burkitt's lymphoma (BL) and Burkitt-like acute lymphoblastic leukemia (B-ALL) and 23 (40%) pts (median age 16.1, 10-50 years) with diffuse large B-cell lymphoma (DLBCL). Treatment corresponded to the original protocol except for use of intermediate doses of the Methotrexate (1 g/sqm i.v. over 36 h) instead of high doses (5 g/sqm i.v. over 24 h). Toxicity estimated for patients of 15 years and upward according to a CTC-NCIC 2.0 scale. Results: CR reached at 85% pts with BL/B-ALL and 79% with DLBCL. 6-years overall survival was 0.71 (SE 0.09) for BL/B-ALL and 0.61 (SE 0.12) for DLBCL. 6-years event free survival (6y-EFS) was 0.69 (SE 0.09) and 0.55 (SE 0.13) relatively. The age of 30 years and upward (6y-EFS 0.17 vs. 0.69; p = 0.027) and Lactate dehydrogenase increase

(6y-EFS 0.49 vs. 1.0; p = 0.023) were adverse prognosis factors by results of the multifactorial analysis (Cox regression). Hematological toxicity of 3-4 degrees developed in most cases: anemia - 26%, granulocytopenia - 40% and thrombocytopenia - 30%. Infectious episodes of 2-3 degrees of toxicity registered in 16 % cases. Mucositis of 3 degrees connected with Methotrexate administrathion have been registered in 12 % blocks of AA and BB. Increase of ALT/AST of 3-4 degrees diagnosed in 16 % cases. The interval between 1 and 2 block of therapy practically has not carried out at patients of 30 years and upward (median 19, range 17-25 days) because of toxicity unlike patients of 15-29 years (median 16, range 14-22 days). Conclusions: our results have proved high efficiency of the revised protocol NHL-BFM-90 for treatment of adolescents and young adults with BL/B-ALL and DLBCL. Failures in treatment of patients of 30 years and upward have confirmed want in other therapy.

RITUXIMAB-CHOP VERSUS CHOP ALONE IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

Mustafa Cetiner, Burhan Ferhanoglu, Fergun Aydin, Nil Molinas, Nukhet Tuzuner, Murat Ozbalak, Isik Kaygusuz

Istanbul Lymphoma Goup-ILeG

Recently, the cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) regimen plus rituximab (R-CHOP) have been used widely to treat patients with newly diagnosed diffuse large B cell lymphoma (DLBCL) and it has also been reported to improve the outcome of DLBCL. We represent a retrospective analysis of newly diagnosed DLBCL patients to evaluate the impact of R-CHOP therapy on response rates. Fifty nine patients with DLBCL between 20-60 years of age (median: 48.0 and mean 51.5 ± 15.3) received 6-8 cycles of R-CHOP. For comparison, DLBCL patients between 21-60 years of age (median: 44.0 and mean 42.9 ± 12.4) who received 6-8 courses of CHOP therapy (n=45) were used as the control group. All patients received classical CHOP (cyclophosphamide 750 mg/m m2, doxorubicin 50 mg/m2, vincristine 1.4 mg/m m2 on day 1 and prednisone 40 mg/m m2 for 5 days) every 4 weeks. In R-CHOP group, rituximab 375 mg/m m2 was administered prior to CHOP chemotherapy. The median follow-up for R-CHOP and CHOP groups were 23.3 ± 11.5 (2-60) and 42.2 ± 32.0 (2-132) months, respectively. The International Prognostic Index (IPI) scores were not significantly different between these groups (median IPI of R-CHOP: 2.0 and mean IPI 1.9 ± 1.2 versus median IPI of CHOP: 2.0 and mean IPI 1.7 ± 1.2). Complete response (CR) and complete unconfirmed response (CuR) rate for R-CHOP was 84.7% (50 of 59 patients) which was statistically significantly higher than CHOP (32 of 45 patients, 71.1%) (p > 0.001). Partial response (PR) rates for R-CHOP and CHOP groups were 5.0% (3 of 59 patients) and 8.8% (4 of 45 patients), respectively. Primary refractory patients was %5 (3 of 59 patients) in the R-CHOP group and 10% (8 of 49 patients) in the CHOP group. Three patients in R-CHOP (%5.0) and 1 patients in CHOP group (%2.2) were died because of treatment related complications. Relapse rates during the follow up period were 24.4 % (11 of 45 patients) for CHOP and 11.8% (7 of 59 patients) for R-CHOP group (p > 0.001). No long-term toxicity appeared to be associated with the addition of rituximab to the CHOP combination. These results also confirmed the benefit of the addition of rituximab to standard CHOP chemotherapy in DLBCL even in young patients under the age of 60.

HIGH LIGHT LECTURE

BURKITT LYMPHOMA: A PARADIGMATIC TUMOUR

Ian Magrath, DSc (Med), FRCP, FRCPath

International Network for Cancer Treatment and Research

The discovery of Burkitt lymphoma (BL) occurred at a time when scientific progress was rapidly accelerating. Heralded by advances in a broad range of disciplines, the last half century has witnessed a revolution in our understanding of the treatment, epidemiology and pathogenesis of cancer. In each of these areas BL has provided an important paradigm that has led to many new discoveries. Burkitt and colleagues provided encouragement to cancer chemotherapy pioneers by demonstrating that several recently developed chemotherapy agents were capable of inducing long term survival in BL. In part based on early combination regimens used in Africa, modern, highly effective therapies have been developed for sporadic BL and large cell lymphomas in children and adolescents, although, unfortunately, most African BL patients still die. The primitive but effective epidemiological studies of Burkitt and colleagues demonstrated a link with malaria and led to the discovery of Epstein-Barr virus (EBV). EBV infects almost the entire human race, but is also associated with a wide range of diseases, both benign and malignant. The study of EBV's survival mechanisms at biological and molecular levels has led to considerable but still incomplete understanding of the pathways that lead to EBV-related diseases in normal and immunodeficient subjects. The discovery of the 8;14 and variant translocations led to the recognition of the central role of c-Myc in BL and to the identification of similar translocations in other lymphoid neoplasms, resulting in the discovery of many genes involved in lymphomagenesis. Recently published gene expression profiles of sporadic BL have shed new light on the cellular origin and definition of BL and related diseases. Genetic signatures may also prove useful in predicting treatment response and improving risk-adaptation of therapy, while EBV genes and genetic abnormalities are likely to provide targets for future, more specific treatment approaches.

Sponsored

ROCHE, Switzerland

NOVARTIS, Switzerland

BIOPTICA, Italy

WELLCOME TRUST, UK

THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE,UK

NATIONAL INSTITUTE OF HEALTH, USA

ZEISS, Germany

MINISTERO DEGLI AFFARI ESTERI, Italy

HUMAN HEALTH FOUNDATION, Italy

JOURNAL OF HEMATOPATHOLOGY, Germany